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QUINE INTELLECTUAL PROPERTY LAW GROUP, P.C.

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Appl. No.	:	10/768,744	Confirmation No. 4909
Applicant	:	Christopher A. Hunter	
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## **APPEAL BRIEF**

### **REAL PARTY IN INTEREST**

The real part of interest in the present appeal is The Trustees of the University of Pennsylvania, the assignee of the above-referenced application.

### **RELATED APPEALS AND INTERFERENCES**

Appellants, Appellants' Attorney, and the assignee of the above-referenced application are unaware of any appeals or interferences that will directly affect, be directly affected by, or have a bearing on, the Board's decision in the present Appeal.

### **STATUS OF CLAIMS**

On May 13, 2011, Appellants appealed from the final rejection of claims 1, 11, 12, 20, 21, 24 to 26, 74 and 75. As originally filed, the case included claims 1 to 72. In the Restriction Action of April 6, 2006, Appellants were requested to elect a group and

species. In the Preliminary Amendment/Restriction Response of September 6, 2006, Appellants elected Group I (claims 1 to 5), and arthritis species for claim 20, with traverse, and added new claim 73. In the first Office Action of November 29, 2006, the Office had been persuaded to prosecute claims 1 to 26 and 73; withdrawing claims 27 to 72 from consideration. In the Response of May 25, 2007, Appellants cancelled claims 2, 4, 5, 7, 9, 10, 14, 16, 17, and 27 to 72. In Appellant's Response and Request for Continued Examination of December 10, 2007, claims 3, 8, 15, 22 and 23 were cancelled. No claims were added or cancelled in Appellant's Response of August 8, 2008. No claims were added or cancelled in Appellant's Response of April 21, 2009. In Appellant's Response of March 15, 2010, claims 6, 13, 18, 19 and 73 were cancelled, and new claims 74 and 75 were added. No claims were added or cancelled in Appellant's Response of November 15, 2010. Accordingly, Appellant believes that claims 1, 11, 12, 20, 21, 24 to 26, 74 and 75 remain pending and under consideration.

#### STATEMENT OF AMENDMENTS

The no claims were amended in the most recent Response of May 13, 2011, to the final Office Action of February 15, 2011. Accordingly, the appealed claims are the claims as provided in the Amendment dated November 15, 2010, as filed in response to the Office Action dated June 16, 2010.

#### SUMMARY OF CLAIMED SUBJECT MATTER

Appellants' invention provides, e.g., a method of treatment by selecting a patient with immune hyperactivity, and administering an effective amount of an IL-27R agonist to the patient. Such methods are described throughout the specification and Figures.

Independent claim 1 is directed to, e.g., methods of treatment by selecting a patient with immune hyperactivity, and administering an effective amount of an IL-27R agonist to the patient, wherein the agonist is selected from the group consisting of IL-27, an active fragment of IL-27, an agonistic antibody, and a IL-27R binding antibody fragment that enhances IL-27R activity. Support for this subject matter can be found, e.g., in a combination of original claims 1 and 3. Further support can be found, e.g., at paragraphs 8, 9, 225, 232 and 237, as published.

Independent claim 24 is directed to, e.g., methods for suppressing a T-helper cell mediated immune response independent of polarization of the immune response in a patient in need thereof. The methods comprise administering to a patient in need an effective amount of an IL-27R agonist. The agonist can be IL-27, an active fragment of IL-27, an agonistic antibody, and a IL-27R binding antibody fragment that enhances IL-27R activity. Support for the T-helper aspect can be found, e.g., at paragraphs 244 to 246. Support for the listed administered ligands can be found, e.g., at paragraphs 8, 9 and 60 of the original specification, and the original claims.

Dependent claims 11, 12, 25 and 26 are essentially in original form, but for changing dependency from cancelled claims. The claims are directed to the nature of T-helper cells involved in the immune hyperactivity. Support for the Th1 and Th2 aspects can be found, e.g., at paragraphs 8, 22, 23, 66 to 69 and 208.

Regarding dependent claims 20 and 22, they are essentially in original form, but for changing dependency from cancelled claims. The claims are directed to the aspect of immune disorders patients may present. Support for the immune disorder aspects can be found, e.g., in the original claims and at paragraphs 66 to 70, 242 and 243.

Regarding dependent claims 74 and 75, are directed to types of hyperactivity mediation. The mediation of the hyperactivity is supported, e.g., at paragraphs 68 to 70.

The appealed claims are set forth in Appendix A.

#### GROUND OF REJECTION TO BE REVIEWED ON APPEAL

In the final Office Action dated February 2, 2011, all pending claims (1, 11, 12, 20, 21, 24 to 26, 74 and 75) were rejected under 35 U.S.C. §102, as allegedly anticipated by any of four cited references. That is, claims 1, 11, 12, 20, 21, 24 to 26, 74 and 75 were rejected under 35 U.S.C. §102(a) as allegedly anticipated by Timans et al. (U.S. 2002/0164609); claims 1, 11, 12, 20, 21, 24 to 26, 74 and 75 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by DeSavage et al. (WO01/029070); claims 1, 11, 12, 20, 21, 24 to 26, 74 and 75 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Bennett et al. (WO97/025425); and 1, 11, 12, 20, 21, 24 to 26, 74 and 75 under 35 U.S.C. §102(e) as allegedly anticipated by Matthews et al. (U.S. 7,074,397).

Appellants request review of these rejections.

Further, the Office has failed to acknowledge the rightful priority date of the provisional application filing dates in this case. Appellants request review of the priority date in this case.

Claims 1, 11, 12, 20, 74 and 75 have been previously provisionally rejected based on alleged obvious-type double patenting related to copending application 11/880,121. However, Appellants believe this issue has been resolved by the filing of a Terminal Disclaimer in the Response of May 13, 2011.

## **ARGUMENT**

### **I. The Priority Claim is Valid to Filed Provisional Applications.**

Benefit of the filing dates of provisional application 60/444,494, filed January 31, 2003, and provisional application 60/519,074, filed November 10, 2003, was denied. As described in detail in the response filed December 10, 2007, and in the response filed August 8, 2008, Applicants have presented a proper priority claim to both documents and respectfully request that priority be acknowledged.

In provisional application 60/444,494, the inventive concept that the receptor for IL-27 is involved in control of the duration and intensity of immune responses in mammals is provided, e.g., in the 2nd column on page 10, where the role of IL-27R is described and proposed as a novel target for immune suppression. Also, page 5, and pages 9-10 further describe finding that the absence of IL-27R leads to immune hyperactivity. One of skill in the art would know based on this data to activate, e.g., with an agonist, IL-27R to suppress the immune system. In fact, the abstract clearly states and the data fully support that the receptor is an "antagonist of T-cell mediated immune hyperactivity." Therefore, with this guidance, one of skill would know, if the receptor antagonizes or blocks immune hyperactivity, to use an agonist or activator of the receptor to suppress the immune system as claimed. These concepts and data are reiterated in the 60/519,074 provisional application, e.g., at page 3, column 2, and page 10, column 2.



With regard to both provisional applications, the claimed invention described is that of using an agonist of IL-27R to suppress the immune system. This is explained in both provisional applications. The key concept embodied in the claimed invention is that activation of IL-27R can be used to suppress the immune system in contrast to the prior art's use of an agonist to activate the immune system. The concept currently in the claims spurred filing of the provisional applications. This concept is fully supported in the specifications of both provisional applications and described in sufficient detail to enable the full scope of the claims. Applicants therefore respectfully request that the benefit claim be acknowledged.

## **II. Rejection of Claims 1, 11, 12, 20, 21, 24 to 26, 74 and 75 Under 35 § 102 is Inappropriate.**

The Final Office Action of February 2, 2011, alleges that claims 1, 11, 12, 20, 21, 24 to 26, 74 and 75 anticipated by Timans et al. (U.S. 2002/0164609), DeSavage et al. (WO01/029070), Bennett et al. (WO97/025425), and Matthews et al. (U.S. 7,074,397). Appellants traversed these rejections and stand by their arguments of record in prior Responses. The following arguments apply to each of the rejected claims 1, 11, 12, 20, 21, 24 to 26, 74 and 75. However, please note the claims do not stand or fall together.

As recently reaffirmed in *Net Moneyin, Inc. v. Verisign, Inc.*, et al. No. 2007-1565, U.S. Court of Appeals for the Federal Circuit, 10/20/2008, 88 U.S.P.Q.2d 1751, to anticipate a claim, the elements of the claim must be in the prior art reference in the same arrangement as in the claim. The Federal Circuit explicitly stated, on page 15, that "Because the hallmark of anticipation is prior invention, the prior art reference—in order to anticipate under 35 U.S.C. § 102—must not only disclose all elements of the claim within the four corners of the document, but must also disclose those elements "arranged as in the claim." *Connell v. Sears, Roebuck & Co.*, 722 F.2d 1542, 1548 (Fed. Cir. 1983)." It further elaborates that "...our precedent informs that the 'arranged as in the claim' requirement applies to all claims and refers to the need for an anticipatory reference to show all of the limitations of the claims arranged or combined in the same way as recited in the claims...."

To anticipate claim 1 and dependents thereto, the prior art must include both of the following steps: (1) a patient having immune hyperactivity must be selected; and (2)

that patient must be administered an effective amount of an IL-27R agonist, which agonist is selected from the group consisting of IL-27, an active fragment of IL-27, an agonistic antibody, and a IL-27R binding antibody fragment that enhances IL-27R activity.

For claim 24 and dependents thereto, the prior art must show a patient needing suppression of a T-helper cell mediated immune response, independent of polarization of the immune response, is administered an effective amount of an IL-27R agonist, wherein said agonist is selected from the group consisting of IL-27, an active fragment of IL-27, an agonistic antibody, and a IL-27R binding antibody fragment that enhances IL-27R activity.

The claims include method step and component aspects. Therefore, to support an anticipation rejection over the claimed invention, it is insufficient for a reference to merely disclose all the compositions involved in the claimed methods, e.g., IL-27, IL-27R and antibodies thereto. The reference must also specify the type of condition for which a patient is being treated and with which compounds that patient is treated.

The present claims are directed to, e.g., the previously unknown, and contradicted, idea that immune hyperactivity can be treated with an Interleukin-27 receptor (IL-27R) agonist. The state of the art, as confirmed in the four cited references for the rejection, was that IL-27R agonists were useful in stimulating hematopoiesis and in fighting obesity and its sequelae. The cited references also confirm that one of skill at the time thought immune hyperactivity problems were best addressed with IL-27R antagonists, as will be discussed below.

With regard to the rejection based on Timans, the Examiner alleges at page 4 of the final Office Action that “Timans teaches the administration of IL-27 in the treatment of immune disorders and inflammation ... see especially paragraph 39.” However, paragraph 39 does not literally or inherently describe treatment of, e.g., inflammation with an IL-27R agonist. Paragraph 39 of Timans is as follows (emphases added):

IL-D80 or IL-27 agonists [note this is not IL-27R agonist], or antagonists, may also act as functional or receptor antagonists. Thus, IL-D80, IL-27, WSX-1/TCCR [aka, IL-27R], or its antagonists, may be useful in the treatment of abnormal medical conditions, including immune disorders, e.g., T cell immune deficiencies,

inflammation, or tissue rejection, or in cardiovascular or neurophysiological conditions.

No data is provided in Timans that could possibly support the idea that all four of these compounds, (p28, IL-27, WSX-1, and WSX-1 antagonists) can or should be used to treat both immune deficiencies and inflammation. This disclosure relied on as a basis of the rejection is illogical, ambiguous, interpreted without regard to the document as a whole, and does not actually or inherently disclose all the limits of the claims.

The paragraph suggests that because IL-27 agonists (not IL-27R agonists) may act as antagonists, a list of peptides, or their antagonists, may be useful in treatment of a list of this or that disorder. How is it to be interpreted? Suppose a reference disclosed birds or fish may also fly or swim using feathers or scales. This does not teach one of skill in the art anything in particular. For example, does this teach that fish fly using feathers; or that birds fly using scales; etc? Such statements are ambiguous and can not teach a specific, without the benefit of impermissible hindsight. At most, one of skill would have to refer to the document as a whole to understand what the author may have meant to say.

If paragraph 39 must be considered, then the teachings of Timans, as a whole, should be considered to help clarify the ambiguities. When Timans discusses IL-27 and IL-27R specifically, the ambiguity is cleared. For example, at paragraphs 160 and 161 of Timans (emphasis added):

Experimental data indicates a possible role for the IL-27 composite cytokine in driving an inflammatory response. ... Taken together the above indicates a role for the composite cytokine [having the IL-27 ligand] and its associated receptor subunit WSX-1/TCCR in inflammatory responses. Therefore antagonizing the function of any of the components in the receptor subunit:ligand complex should have a beneficial effect in inflammatory diseases, e.g., inflammatory bowel disease, rheumatoid arthritis, etc.

As discussed above, it is not clear from reading paragraph 39 whether IL-27R agonists “or” antagonists reduce inflammation. However Timmons further expounds at paragraphs 132, 135 and 161 to confirm the specific teaching (contrary to the present claims) that antagonists,

not agonists, are expected to reduce inflammation (which can include aspects of immune hyperactivity). This is also what one of skill would have expected, based on the state of knowledge at the time (see, state of knowledge reviewed starting at page 9 of the March 15, 2010 Response).

Further, the statement of paragraph 39 can not be said to teach the claims because the premise of the argument does not support the conclusion. That is, because agonists or antagonists of IL-27 may have certain functions, it is not logical to conclude that it is useful to treat with IL-27 itself. Such a statement does not logically lead to the conclusion that IL-27R agonists would be useful against immune hyperactivity. Because the statement of paragraph 39 is illogical on its face, it can not stand as a basis for the rejection.

Moreover, assuming the statement were logical (and it is not), it still does not inherently disclose the specific relationships of the claimed methods. See, e.g., *Ex parte Levy*, 17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990), *Continental Can Co. USA v. Monsanto Co.*, 948 F.2d 1264, 1268, 20 USPQ2d 1746, 1749 (Fed. Cir. 1991), and *In re Oelrich*, 666 F.2d 578, 581 - 82, 212 USPQ 323, 326 (CCPA 1981), wherein allegedly inherent characteristic must necessarily flow in every case from the teachings of the applied prior art. That is, e.g., a statement suggesting that this or that peptide may treat disease 1 or disease 2, does not specify which peptide may treat which disease. Even as a generic concept, it does not disclose a specific lesson. At best, the statement suggests something that may be looked in to. The statement does not inherently teach the claimed methods because the statement does not necessarily arrive at the present claims in every case. Here, particular peptides may be associated with the treatment cited in hindsight, or it may be associated with a different treatment. Because a peptide may be associated with a treatment outside the present methods, it does not necessarily (inherently) describe the claimed methods.

Because the key cited statement of Timans is illogical, one of skill can not learn even a generic concept from paragraph 39. Ignoring the lack of foundation, the statement still does not inherently disclose the specific limitations of the claims. Further, reviewing Timans for an explanation of the illogical and ambiguous statement of paragraph 39, one finds an explanation that confirms Timans' directs one of skill to understand, e.g., immune hyperactivity should be treated with antagonists, as was the common knowledge at

the time, contrary to the methods of the claims. Further, because Timans is not prior art (with recognition of the rightful priority claim) to the present application, it can not anticipate the claims. Because Timans fails to anticipate the present claims for any number of reasons, Appellants respectfully request the rejection be withdrawn.

With regard to the rejection based on DeSavage, the Examiner allegedly finds the present claims in the statement at Part 11 (page 59, starting at line 27; paragraph 344, as published) of DeSavage:

It is contemplated that the polypeptides, antibodies and other active compounds of the present invention may be used to treat various immune related diseases and conditions, such as T cell mediated diseases, including those characterized by infiltration of inflammatory cells into a tissue, stimulation of T-cell proliferation, inhibition of T-cell proliferation, increased or decreased vascular permeability or the inhibition thereof.

What are “the active compounds of the present invention” in DeSavage? The claimed invention in DeSavage is a method of enhancing, stimulating or potentiating the differentiation of T-cells into the Th2 subtype instead of the Th1 subtype, comprising contacting said T-cells with an effective amount of a TCCR [IL-27R] antagonist. See DeSavage, claim 1. The DeSavage compounds are antagonists, not agonists of the IL-27R.

Further evidence identifying the DeSavage active compounds can be found throughout the reference. The active compounds are all antagonists. In the section entitled Uses for Anti-TCCR Antibodies, starting at paragraph 331, for example, such antibodies are never described as useful therapeutics. The antibodies are only said to be useful as diagnostic reagents and in affinity purifications. IL-27 is never mentioned in DeSavage.

Other citations in the rejection seem irrelevant. For example, the statements in the Abstract and at page 63, line 36, speak in generalities and fail to suggest to one of skill, e.g., administration of the specific IL-27R agonists to a patient with immune hyperactivity. That is, e.g., a statement that “inhibition of molecules with proinflammatory properties may have therapeutic benefit,” does not anticipate the claims.

In short, DeSavage may employ TCCR antagonists against inflammation, but DeSavage never suggests agonists may be beneficial. Further, among the extensive list of active compounds, none overlap those specifically listed in the claims as agonists for treatment of immune hyperactivity. Because DeSavage does not disclose all limitations of the claims, Appellants respectfully request withdrawal of the rejections for alleged anticipation.

With regard to the rejection based on Bennett, the Examiner allegedly finds disclosure of the present claims in the statements of Bennett at pages 4 and 5 that agonist antibodies “are useful for activating the WSX receptor for *in vitro* uses whereby enhancement of proliferation and/or differentiation of a cell comprising the receptor is desired.” The paragraph goes on to say “these antibodies may be used to treat conditions in which an effective amount of WSX receptor activation leads to a therapeutic benefit”, as cited in the rejection. Bennett goes on to clarify that WSX agonist antibodies function in decreasing obesity (and indirectly the sequelae of obesity) and in hematopoiesis. However, IL-27 is not ever mentioned in Bennett. Although Bennett lists a large number of indications for agonist treatment, this is in the context of immune stimulation benefits for those conditions. That is, Bennett never discloses patients with immune hyperactivity would benefit from agonist treatment. In fact, one of skill would understand the opposite from reading Bennett.

If a piece of prior art does not expressly disclose a limitation, there can be anticipation only if a person of ordinary skill in the art would understand the prior art to disclose the limitation and could combine the prior art description with his own knowledge to make the claimed invention. See, *Helifix Ltd. v. Blok-Lok, Ltd.*, 208 F.3d 1339, 1347 [54 USPQ2d 1299] (Fed. Cir. 2000). Here, Bennett discloses immune activity stimulation from IL-27R agonists and this was the understanding of one of skill in the art at the time. Therefore, Bennett can not be considered to disclose the present methods.

At page 7 of the final Office Action, the Examiner attempts to link agonist antibody administration with immune hyperactivity. However, this fails because the conditions are disclosed only as conditions requiring enhanced immune activity or reduction

of obesity. That is, Bennett teaches only agonist antibody treatments activating immune systems, not the opposite methods of treating a patient having immune hyperactivity.

For example, the key citations of treatments with antibody agonists at page 5 (e.g., line 25) of Bennett are directed to methods "for treating the medical sequelae of obesity" including sequelae having some overlap with immune disorders listed in present claim 21 (discussed below). This does not prompt one of skill to select patients with immune hyperactivity for treatment. Further note, the overlapping sequelae are not disclosed as directly treated by reduction of immune hyperactivity, but, e.g., as benefiting from reduction of the causative obesity. Further evidence is at page 56, line 16, where diabetes treatment is not by reduction of an immune hyperactivity, but by restoration of "insulin sensitivity in patients." The treatments of obesity sequelae are not expressly disclosed or inherently based on immune activity reduction.

Elsewhere, "the invention provides a method for stimulating proliferation and/or differentiation of a cell [e.g.,] hematopoietic precursor, e.g., a CD34+ cell." Bennett discusses immune stimulation and proliferation treatments, but never the opposite problem of reducing immune hyperactivity with WSX agonist antibodies. For example, at page 6, line 11, treatment is to "benefit from an increase in various blood cells"; and at line 15, "benefit from enhancement of lymphopoiesis [including a list of ailments, such as SLE] and other disorders characterized by lymphocyte deficiency." Emphasis added.

At line 27, a large listing is provided of "disorders in which an increase in erythropoiesis may be beneficial." Contrary to the methods of the claims, all of the listed medical conditions cited by the Examiner as present in dependent claim 21 are disclosed in Bennett as requiring additional immune activity for treatment, not reduced activity as in the claims. Therefore, Bennett can not reasonably be interpreted as teaching one of skill to select of patients with immune hyperactivity, as required for a rejection. Nor can it be suggested that Bennett teaches one of skill to administer an effective amount of agonist to treat immune hyperactivity. To the contrary, Bennett teaches the opposite.

Appellants note that medical disorders present diverse modes of pathology and symptoms. The various symptoms can result from different interactions between the patient and pathological agent. This is why, e.g., a patient with one condition often requires

a variety of drugs to treat all the symptoms. That is, a patient with a disorder presenting multiple symptoms or conditions thought separately to benefit immune stimulation and also immune moderation. Patients are often prescribed conflicting medicines to address different symptoms of a disorder. For example, in inflammation from an infection, or in allergic response to a parasite, a patient can benefit optionally from either immune moderation to reduce the swelling and itching and/or immune stimulation to directly attack the pathogen. Therefore, just because disorders cited as benefiting from immune stimulation by agonists in Bennett happen to overlap with, e.g., disorders benefiting from reduction of immune hyperactivity in dependent claim 21 does not show that Bennett disclosed reducing immune hyperactivity of patients with IL-27R agonists. To the contrary.

Because Bennett does not disclose to one of skill in the art 1) that IL-27R agonists reduce immune hyperactivity, 2) the idea of selecting a patient with immune hyperactivity for treatment of symptoms with an IL-27R agonist, or 3) administering an amount of IL-27R agonist in treatment of such a hyperactivity, the claims can not be considered anticipated by Bennett.

With regard to the rejection based on Matthews, again the disclosure directs one of skill in the art to employ agonist antibodies to "stimulate proliferation of hematopoietic stem cells", not to treat patients having immune hyperactivity. As with Bennett, Matthews only teaches the then common knowledge that WSX stimulates blood cell proliferation (column 44, line 25) and is useful in fighting obesity (column 50, line 49).

At page 8 of the final Office Action the Examiner presents a long list of citations allegedly responding to Appellants' request that "the examiner point out where ... an agonist ... of IL-27R ... is mentioned specifically in connection with treating immune hypersensitivity." Appellants appreciate that the Examiner finally presented the long requested specific citations. However, careful review of each citation shows Matthews teaching one of skill in the art IL-27R agonists employed to stimulate immune responses and activity not to, e.g., treat a problem identified as resulting from immune hyperactivity. Matthews discloses selecting patients requiring a boost in immune activity, not selecting patients with immune hyperactivity, as required in the claims.



For example, cited column 3, line 38, discusses methods of "stimulating proliferation ... of cells." Column 3, line 47, discloses binding of agonist antibody in the context of proliferation of lymphoid and myeloid cells. Column 14, line 61 discloses only that agonist antibody activates WSX. Column 17, line 14, discloses "agonist antibodies, may be used to stimulate proliferation ..." Column 44, line 22, discloses utilities of WSX receptor antibodies, including induction of proliferation and affinity purification. There is no column 80 in Matthews. Column 45, line 36, discloses only receptor antibody preparation. The Examiner has only presented disclosures that direct one of skill in the art to understand that WSX receptor agonist antibodies stimulate immune systems. Reading the citations of the Examiner, one of skill would be led to understand that WSX receptor agonists are useful in stimulating immune response and to select patients having, e.g., immune hypoactivity for treatment.

As with Bennett, there may be slight coincidental overlap between the large lists of disorders in the reference and dependent claim 21. However, as discussed above, diseases are complex and may have a variety of causes and symptoms that can be treated by a variety of therapeutics, some having conflicting modes of operation (as is well known in the pharmaceutical arts). Yet, this does not change the fact that the disclosure of Matthews leads one of skill in the art to understand anti-WSX agonists are useful in immune stimulation and that patents having hyp immunity problems would benefit from treatment.

Because Matthews does not teach at least the claim limitations of selecting a patient having immune hyperactivity, of administering agonists in an amount effective in treating the hyperactivity, the claims are not anticipated by Matthews.

CONCLUSION

Applicants submit that the Examiner's rejection of claims 1, 11, 12, 20, 21, 24 to 26, 74 and 75 is improper. Withdrawal of these rejections by the Examiner or reversal of the rejections by the Board is respectfully requested.

The Commissioner is authorized to charge the fee under 37 C.F.R. §1.17(c) and any other required fees, or to credit any overpayments, to Deposit Account 50-0893.

If a telephone conference would expedite prosecution of the above-identified application, the Examiner is invited to phone the undersigned at (510) 769-3510.

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Attachments:

- 1) Appendix A – Appealed Claims for 10/768,744;
- 2) Appendices B and C

**APPENDIX A**  
**APPEALED CLAIMS FOR 10/768,744**

**1 (Previously Presented).** A method of treating a patient, which comprises:

selecting a patient with immune hyperactivity; and

administering to said patient an effective amount of an IL-27R agonist, wherein said agonist is selected from the group consisting of IL-27, an active fragment of IL-27, an agonistic antibody, and a IL-27R binding antibody fragment that enhances IL-27R activity.

**2-10 (Cancelled).**

**11 (Previously Presented).** The method of claim **74**, wherein said T-helper cell is Th1.

**12 (Previously Presented).** The method of claim **74**, wherein said T-helper cell is Th2.

**13-19 (Cancelled).**

**20 (Previously Presented).** The method of claim **1**, wherein said patient has an immune disorder selected from the group consisting of autoimmune disorders, hypersensitivity disorders, allergies, and asthma.

**21 (Previously Presented).** The method of claim **20**, wherein said immune disorder is selected from the group consisting of: acute pancreatitis; Addison's disease; alcohol-induced liver injury; Alzheimer's disease; amyotrophic lateral sclerosis; asthma; pulmonary diseases; atherosclerosis; autoimmune vasculitis; autoimmune hepatitis-induced hepatic injury; cachexia/anorexia; AIDS-induced cachexia; multiple myeloma; leukemia; myelogenous leukemia; tumor metastasis; chronic fatigue syndrome; congestive heart failure; coronary restenosis; myocardial dysfunction; a coronary artery bypass graft associated condition; juvenile onset Type 1 diabetes; diabetes mellitus insulin resistance; endometriosis; endometritis; endometriosis/endometritis related condition; epididymitis; erythropoietin resistance; fever; fibromyalgia; analgesia; glomerulonephritis; graft versus host disease/transplant rejection; Graves' disease; Guillain-Barre syndrome; Hashimoto's disease; hemolytic anemia; hemorrhagic shock; hyperalgesia; inflammatory bowel disease; ulcerative colitis; Crohn's disease; an inflammatory condition of a joint; rheumatic diseases; osteoarthritis; rheumatoid arthritis; juvenile (rheumatoid) arthritis; seronegative polyarthritis; ankylosing spondylitis; Reiter's syndrome; reactive arthritis; Still's disease; enteropathic arthritis; polymyositis; dermatomyositis; scleroderma; systemic sclerosis; cerebral vasculitis; Lyme disease; staphylococcal induced arthritis; Sjogren's syndrome; rheumatic fever;

polychondritis; polymyalgia rheumatica; giant cell arteritis; inflammatory eye disease; corneal transplant associated inflammatory eye disease; inflammatory bowel disease; Kawasaki's disease; lung disease; lupus nephritis; multiple sclerosis; myasthenia gravis; myopathiceneuroinflammatory disease; uveitis; osteoporosis; Parkinson's disease; pemphigus; Pityriasis rubra pilaris; prostatitis; a prostatitis related conditions; psoriasis; a psoriasis related condition; psoriatic arthritis; pulmonary fibrosis; reperfusion injury; sarcoidosis; scleroderma; septic shock; sleep disturbance; spondyloarthropathies; systemic lupus erythematosus; temporal mandibular joint disease; thyroiditis; tissue transplantation; an inflammatory condition resulting from strain; an inflammatory condition resulting from sprain; an inflammatory condition resulting from cartilage damage; an inflammatory condition resulting from trauma; an inflammatory condition resulting from orthopedic surgery; an inflammatory condition resulting from infection; transplant rejection; and vasculitis.

**22 and 23 (Cancelled).**

**24 (Previously Presented).** A method for suppressing a T-helper cell mediated immune response independent of polarization of the immune response in a patient in need thereof, which comprises administering to said patient an effective amount of an IL-27R agonist, wherein said agonist is selected from the

group consisting of IL-27, an active fragment of IL-27, an agonistic antibody, and a IL-27R binding antibody fragment that enhances IL-27R activity.

**25 (Previously Presented).** The method of claim **24**, wherein said T-helper cell is Th1.

**26 (Previously Presented).** The method of claim **24**, wherein said T-helper cell is Th2.

**27 to 73 (Cancelled).**

**74. (Previously Presented)** The method of claim **1**, wherein the immune hyperactivity is T-helper cell mediated immune hyperactivity.

**75. (Previously Presented)** The method of claim **1**, wherein the immune hyperactivity is interferon- $\gamma$  mediated immune hyperactivity.

**APPENDIX C**  
**RELATED PROCEEDINGS**

(none)

**APPENDIX B**  
**EVIDENCE APPENDIX**

- De Sauvage et al., (WO01/029070)
- Bennet at al., (WO97/025425)





## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US97/00325 <b>(22) International Filing Date:</b> 7 January 1997 (07.01.97)  <b>(30) Priority Data:</b> 08/585,005           8 January 1996 (08.01.96)       US 08/667,197           20 June 1996 (20.06.96)       US  <b>(71) Applicant:</b> GENENTECH, INC. [US/US]; 460 Point San Bruno Boulevard, South San Francisco, CA 94080-4990 (US).  <b>(72) Inventors:</b> BENNETT, Brian; 1332 Oddstat Boulevard, Pacifica, CA 94044 (US). CARTER, Paul, J.; 2074 18th Avenue, San Francisco, CA 94116 (US). CHIANG, Nancy, Y.; 622 Quintara Street, San Francisco, CA 94116 (US). KIM, Kyung, Jin; 622 Benvenue Avenue, Los Altos, CA 94024 (US). MATTHEWS, William; 560 Summit Springs Road, Woodside, CA 94062 (US). RODRIGUES, Maria, L.; 2320 Donegal Avenue, South San Francisco, CA 94080 (US).  <b>(74) Agents:</b> HASAK, Janet, E. et al.; Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, CA 94080-4990 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> WSX RECEPTOR AND LIGANDS  <b>(57) Abstract</b> <p>The WSX receptor and antibodies which bind thereto (including agonist and neutralizing antibodies) are disclosed, including various uses therefor. Uses for WSX ligands (e.g., anti-WSX receptor agonist antibodies or OB protein) in hematopoiesis are also disclosed.</p>		

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**WSX RECEPTOR AND LIGANDS****CROSS REFERENCES**

This application is a continuation-in-part of co-pending U.S. Application Serial No. 08/667,197 filed June 20, 1996, which is a continuation-in-part of co-pending U.S. Application Serial No. 08/585,005 filed January 8, 1996, which applications are incorporated herein by reference and to which applications priority is claimed under 35 USC §120.

**BACKGROUND OF THE INVENTION****Field of the Invention**

The present invention pertains generally to the WSX receptor and ligands and uses for these molecules.

**10 Description of Related Art****A. HEMATOPOIESIS**

The process of blood cell formation whereby red and white blood cells are replaced through the division of cells located in the bone marrow is called hematopoiesis. For a review of hematopoiesis see Dexter and Spooncer (*Ann. Rev. Cell Biol.* 3:423-441 (1987)).

15 There are many different types of blood cells which belong to distinct cell lineages. Along each lineage, there are cells at different stages of maturation. Mature blood cells are specialized for different functions. For example, erythrocytes are involved in O<sub>2</sub> and CO<sub>2</sub> transport; T and B lymphocytes are involved in cell and antibody mediated immune responses, respectively; platelets are required for blood clotting; and the granulocytes and macrophages act as general scavengers and accessory cells. Granulocytes can be further divided into  
20 basophils, eosinophils, neutrophils and mast cells.

Each of the various blood cell types arises from pluripotent or totipotent stem cells which are able to undergo self-renewal or give rise to progenitor cells or Colony Forming Units (CFU) that yield a more limited array of cell types. As stem cells progressively lose their ability to self-renew, they become increasingly lineage restricted. It has been shown that stem cells can develop into multipotent cells (called "CFC-Mix" by Dexter and  
25 Spooncer, *supra*). Some of the CFC-Mix cells can undergo renewal whereas others lead to lineage-restricted progenitors which eventually develop into mature myeloid cells (*e.g.*, neutrophils, megakaryocytes, macrophages and basophils). Similarly, pluripotent stem cells are able to give rise to PreB and PreT lymphoid cell lineages which differentiate into mature B and T lymphocytes, respectively. Progenitors are defined by their progeny, *e.g.*, granulocyte/macrophage colony-forming progenitor cells (GM-CFU) differentiate into neutrophils or  
30 macrophages; primitive erythroid burst-forming units (BFU-E) differentiate into erythroid colony-forming units (CFU-E) which give rise to mature erythrocytes. Similarly, the Meg-CFU, Eos-CFU and Bas-CFU progenitors are able to differentiate into megakaryocytes, eosinophils and basophils, respectively.

Hematopoietic growth factors (reviewed in Andrea, *NEJM* 330(12):839-846 (1994)) have been shown to enhance growth and/or differentiation of blood cells via activation of receptors present on the surface of blood  
35 progenitor cells of the bone marrow. While some of these growth factors stimulate proliferation of restricted lineages of blood cells, others enhance proliferation of multiple lineages of blood cells. For example, erythropoietin (EPO) supports the proliferation of erythroid cells, whereas interleukin-3 (IL-3) induces proliferation of erythroid and myeloid lineages and is therefore considered a multi-lineage factor.

In recent years, several hematopoietic growth factor receptors have been isolated. Due to their low abundance and their existence in both high-affinity and low-affinity forms, biochemical characterization of these receptors has been hampered.

Cytokine receptors frequently assemble into multi-subunit complexes. Sometimes, the  $\alpha$  subunit of this complex is involved in binding the cognate growth factor and the  $\beta$ -subunit may contain an ability to transduce a signal to the cell. These receptors have been assigned to three subfamilies depending on the complexes formed. Subfamily 1 includes the receptors for erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), interleukin-4 (IL-4), interleukin-7 (IL-7), growth hormone (GH) and prolactin (PRL). Ligand binding to receptors belonging to this subfamily is thought to result in homodimerization of the receptor. Subfamily 2 includes receptors for IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-5 (IL-5), interleukin-6 (IL-6), leukemia inhibitory factor (LIF), oncostatin M (OSM) and ciliary neurotrophic factor (CNTF). Subfamily 2 receptors are heterodimers having an  $\alpha$ -subunit for ligand binding and  $\beta$ -subunit (either the shared  $\beta$ -subunit of the IL-3, GM-CSF and IL-5 receptors or the gp130 subunit of the IL-6, LIF, OSM and CNTF receptors) for signal transduction. Subfamily 3 contains only the interleukin-2 (IL-2) receptor. The  $\beta$  and  $\gamma$  subunits of the IL-2 receptor complex are cytokine-receptor polypeptides which associate with the  $\alpha$ -subunit of the unrelated Tac antigen.

#### B. OBESITY

Obesity is the most common nutritional disorder which, according to recent epidemiologic studies, affects about one third of all Americans 20 years of age or older. Kuczmarski *et al.*, *J. Am. Med. Assoc.* 272:205-11 (1994). Obesity is responsible for a variety of serious health problems, including cardiovascular disorders, type II diabetes, insulin-resistance, hypertension, hypertriglyceridemia, dyslipoproteinemia, and some forms of cancer. Pi-Sunyer, F., *Anns. Int. Med.* 119: 655-60 (1993); Colfitz, G., *Am. J. Clin. Nutr.* 55:503S-507S (1992). A single-gene mutation (the obesity or "ob" mutation) has been shown to result in obesity and type II diabetes in mice. Friedman, *Genomics* 11:1054-1062 (1991).

Zhang *et al.*, *Nature* 372:425-431 (1994) have recently reported the cloning and sequencing of the mouse *ob* gene and its human homologue, and suggested that the *ob* gene product, leptin or OB protein, may function as part of a signalling pathway from adipose tissue that acts to regulate the size of the body fat depot. Parabiosis experiments performed more than 20 years ago predicted that the genetically obese mouse containing two mutant copies of the *ob* gene (*ob/ob* mouse) does not produce a satiety factor which regulates its food intake, while the diabetic (*db/db*) mouse produces but does not respond to a satiety factor. Coleman and Hummal, *Am. J. Physiol.* 217:1298-1304 (1969); Coleman, *Diabetol* 9:294-98 (1973). Recent reports by three independent research teams have demonstrated that daily injections of recombinant OB protein inhibit food intake and reduce body weight and fat in grossly obese *ob/ob* mice but not in *db/db* mice (Pellemounter *et al.*, *Science* 269:540-43 (1995); Halaas *et al.*, *Science* 269:543-46 (1995); Campfield *et al.*, *Science* 269: 546-49 (1995)), suggesting that the OB protein is such a satiety factor as proposed in early cross-circulation studies.

Researchers suggest that at least one OB receptor is localized in the brain. The identification and expression cloning of a leptin receptor (OB-R) was reported by Tartaglia *et al.* *Cell* 83:1263-71 (1995). Various isoforms of a OB receptor are described by Cioffi *et al.* *Nature* 2:585-89 (1996). See, also, WO 96/08510.

The mouse *db* gene has recently been cloned (Lee *et al.* *Nature* 379:632 (1996) and Chen *et al.* *Cell* 84:491-495 (1996)). Previous data had suggested that the *db* gene encoded the receptor for the obese (*ob*) gene product, leptin (Coleman *et al.*, *Diabetologia* 9:294-8 (1973) and Coleman *et al.*, *Diabetologia* 14:141-8 (1978)). It has been very recently confirmed that the *db/db* mouse results from a truncated splice variant of the OB receptor which likely renders the receptor defective in signal transduction (Lee *et al.*, *Nature* 379:632 (1996) and Chen *et al.*, *Cell* 84: 491-495 (1996)).

#### SUMMARY OF THE INVENTION

The invention herein is concerned with the WSX cytokine receptor and a soluble form of the receptor which is the WSX receptor extracellular domain (ECD). The WSX receptor polypeptides are optionally  
10 conjugated with, or fused to, molecules which increase the serum half-lives thereof and can be formulated as pharmaceutical compositions comprising the polypeptide and a physiologically acceptable carrier.

In certain embodiments, the WSX receptor ECD may be used as an antagonist insofar as it may bind to WSX ligand and thereby reduce activation of endogenous WSX receptor. This may be useful in conditions characterized by excess levels of WSX ligand and/or excess WSX receptor activation in a mammal. WSX  
15 receptor ECD may, for example, be used to treat metabolic disorders (*e.g.*, anorexia or steroid-induced truncalobesity), stem cell tumors and other tumors which express WSX receptor.

Pharmaceutical compositions of the WSX receptor ECD may further include a WSX ligand. Such dual compositions may be beneficial where it is therapeutically useful to prolong the half-life of WSX ligand and/or activate endogenous WSX receptor directly as a heterotrimeric complex.

The invention also relates to chimeric WSX receptor molecules, such as WSX receptor immunoadhesins  
20 (having long half-lives in the serum of a patient treated therewith) and epitope tagged WSX receptor. Immunoadhesins may be employed as WSX receptor antagonists in conditions or disorders in which neutralization of WSX receptor biological activity may be beneficial. Bispecific immunoadhesins (combining a WSX receptor ECD with a domain of another cytokine receptor) may form high affinity binding complexes  
25 for WSX ligand.

The invention further provides methods for identifying a molecule which binds to and/or activates the WSX receptor. This is useful for discovering molecules (such as peptides, antibodies, and small molecules) which are agonists or antagonists of the WSX receptor. Such methods generally involve exposing an immobilized WSX receptor to a molecule suspected of binding thereto and determining binding of the molecule  
30 to the immobilized WSX receptor and/or evaluating whether or not the molecule activates (or blocks activation of) the WSX receptor. In order to identify such WSX ligands, the WSX receptor may be expressed on the surface of a cell and used to screen libraries of synthetic compounds and naturally occurring compounds (*e.g.*, endogenous sources of such naturally occurring compounds, such as serum). The WSX receptor can also be used as a diagnostic tool for measuring serum levels of endogenous WSX ligand.

In a further embodiment, a method for purifying a molecule which binds to the WSX receptor is  
35 provided. This can be used in the commercial production and purification of therapeutically active molecules which bind to this receptor. In the method, the molecule of interest (generally a composition comprising one or more contaminants) is adsorbed to immobilized WSX receptor (*e.g.*, WSX receptor immunoadhesin immobilized on a protein A column). The contaminants, by virtue of their inability to bind to the WSX receptor, will

generally flow through the column. Accordingly, it is then possible to recover the molecule of interest from the column by changing the elution conditions, such that the molecule no longer binds to the immobilized receptor.

In further embodiments, the invention provides antibodies that specifically bind to the WSX receptor. Preferred antibodies are monoclonal antibodies which are non-immunogenic in a human and bind to an epitope  
5 in the extracellular domain of the receptor. Preferred antibodies bind the WSX receptor with an affinity of at least about  $10^6$  L/mole, more preferably  $10^7$  L/mole.

Antibodies which bind to the WSX receptor may optionally be fused to a heterologous polypeptide and the antibody or fusion thereof may be used to isolate and purify WSX receptor from a source of the receptor.

In a further aspect, the invention provides a method for detecting the WSX receptor *in vitro* or *in vivo*  
10 comprising contacting the antibody with a sample suspected of containing the receptor and detecting if binding has occurred. Based on the observation herein that CD34+ cells possess WSX receptor, use of WSX antibodies for identification and/or enrichment of stem cell populations (in a similar manner to that in which CD34 antibodies are presently used) is envisaged.

For certain applications, it is desirable to have an agonist antibody which can be screened for as  
15 described herein. Such agonist antibodies are useful for activating the WSX receptor for *in vitro* uses whereby enhancement of proliferation and/or differentiation of a cell comprising the receptor is desired. Furthermore, these antibodies may be used to treat conditions in which an effective amount of WSX receptor activation leads to a therapeutic benefit in the mammal treated therewith. For example, the agonist antibody can be used to enhance survival, proliferation and/or differentiation of a cell comprising the WSX receptor. In particular, agonist  
20 antibodies and other WSX ligands may be used to stimulate proliferation of stem cells/progenitor cells either *in vitro* or *in vivo*. Other potential therapeutic applications include the use of agonist antibodies to treat metabolic disorders (such as obesity and diabetes) and to promote kidney, liver or lung growth and/or repair (*e.g.*, in renal failure).

For therapeutic applications it is desirable to prepare a composition comprising the agonist antibody  
25 and a physiologically acceptable carrier. Optionally, such a composition may further comprise one or more cytokines.

In other embodiments, the antibody is a neutralizing antibody. Such molecules can be used to treat conditions characterized by unwanted or excessive activation of the WSX receptor.

In addition to the above, the invention provides isolated nucleic acid molecules, expression vectors and  
30 host cells encoding the WSX receptor which can be used in the recombinant production of WSX receptor as described herein. The isolated nucleic acid molecules and vectors are also useful for gene therapy applications to treat patients with WSX receptor defects and/or to increase responsiveness of cells to WSX ligand.

This application also relates to agonist antibodies which specifically bind to the WSX receptor and mimic one or more biological activities of naturally occurring WSX ligand, OB protein. Preferred antibodies are  
35 those with a strong binding affinity for human WSX receptor (*e.g.* having a  $K_d$  of no more than about  $1 \times 10^8$  M; and preferably no more than about  $1 \times 10^9$  M). In preferred embodiments, the agonist antibody binds to both human and murine WSX receptor.

Antibodies with defined agonistic activity in a bioassay, the KIRA ELISA, are disclosed herein. Preferred antibodies have an IC<sub>50</sub> in the KIRA ELISA of about 0.5 µg/ml or less, preferably about 0.2 µg/ml or less, and most preferably about 0.1 µg/ml or less.

5 The agonist antibodies of interest herein may have one or more of the biological characteristics of antibody 2D7, 1G4, 1E11 or 1C11 (see Example 13) or clones 3, 4, or 17 (see Example 14). For example, the antibody may bind to the epitope bound by any one of these antibodies, and/or may have some or all of the hypervariable region residues of these antibodies.

10 The agonist antibody may be one which decreases body weight and/or fat-depot weight and/or food intake in an obese mammal (e.g. in an *ob/ob* mouse). The preferred agonist antibody is one which exerts an adipose-reducing effect in an obese mammal (e.g. an *ob/ob* mouse) which is in excess of that induced by a reduction in food intake (Levin *et al. Proc. Natl. Acad. Sci. USA* 93:1726-1730 (1996)).

The agonist antibody may also have the property of inducing differentiation and/or proliferation and/or survival of hematopoietic progenitor cells. For example, the agonist antibody may induce lymphopoiesis, erythropoiesis and/or myelopoiesis.

15 The invention further provides a composition comprising the agonist antibody and a physiologically acceptable carrier. The composition for therapeutic use is sterile and may be lyophilized. For use in hematopoiesis, for example, the composition may further comprise a cytokine.

20 In another aspect, the invention provides a method for activating the WSX receptor which comprises exposing the WSX receptor to an amount of an agonist anti-WSX receptor antibody which is effective for activating the WSX receptor. The invention further provides a method for enhancing proliferation and/or differentiation of a cell which expresses the WSX receptor at its cell surface comprising exposing the cell to an amount of exogenous agonist anti-WSX receptor antibody which is effective for enhancing proliferation and/or differentiation of the cell. In another embodiment, the invention provides a method for decreasing body weight and/or fat-depot weight and/or food intake in an obese mammal (e.g. a human) comprising administering an effective amount of the agonist antibody to the mammal. Also, the invention provides a method for treating the medical sequelae of obesity in a mammal, such as, e.g., arteriosclerosis, Type II diabetes, polycystic ovarian disease, cardiovascular diseases, osteoarthritis, dermatological disorders, hypertension, insulin resistance, hypercholesterolemia, hypertriglyceridemia, cancer and cholelithiasis, comprising administering an effective amount of an agonist anti-WSX receptor antibody to the mammal. The mammal to be treated may be one diagnosed with any one or more of these diseases, or may be predisposed to these diseases.

25 In another aspect, the present invention pertains to the discovery herein that WSX ligands, such as obesity (OB) protein, play a role in hematopoiesis via signalling through the WSX receptor. The role of the WSX receptor-ligand signalling pathway appears to be at the level of the early hematopoietic precursor as is evident by the ability of OB protein to simulate myelopoiesis, erythropoiesis (e.g. splenic erythropoiesis) and most dramatically, lymphopoiesis. Accordingly, WSX ligands can be used to stimulate proliferation and/or differentiation and/or survival of hematopoietic progenitor cells either *in vitro* or *in vivo* (e.g. for treating hematopoietic diseases or disorders).

35 Thus, the invention provides a method for stimulating proliferation and/or differentiation of a cell which expresses the WSX receptor (especially the WSX receptor variant 13.2, which is demonstrated herein to have

the capacity to transmit a proliferative signal) at its cell surface comprising the step of contacting the WSX receptor with an amount of WSX ligand which is effective for stimulating proliferation and/or OB protein differentiation of the cell. In preferred embodiments, the cell which is exposed to the WSX ligand is a hematopoietic precursor, *e.g.* a CD34+ cell. The WSX ligand may be OB protein or an agonist antibody which  
5 binds to the WSX receptor. For *in vivo* use, the WSX ligand of choice may be a long half-life derivative of an OB protein, such as OB-immunoglobulin chimera and/or OB protein modified with a nonproteinaceous polymer, such as polyethylene glycol (PEG). The method contemplated herein may lead to an increase in the proliferation and/or differentiation of lymphoid, myeloid and/or erythroid blood cell lineages and encompasses both *in vitro* and *in vivo* methods. For *in vitro* uses, the cell possessing the WSX receptor may be present in cell culture. As  
10 to *in vivo* methods, the cell may be present in a mammal, especially a human (*e.g.* one who is suffering from decreased blood levels and who could benefit from an increase in various blood cells). Potential patients include those who have undergone chemo- or radiation therapy, or bone marrow transplantation therapy. Thus, the invention provides a method for repopulating blood cells (*e.g.* erythroid, myeloid and/or lymphoid blood cells) in a mammal comprising administering to the mammal a therapeutically effective amount of a WSX ligand.

15 Mammals which may benefit from an enhancement of lymphopoiesis include those predisposed to, or suffering from, any one or more of the following exemplary conditions: lymphocytopenia; lymphorrhea; lymphostasis; immunodeficiency (*e.g.* HIV and AIDS); infections (including, for example, opportunistic infections and tuberculosis (TB)); lupus; and other disorders characterized by lymphocyte deficiency. An effective amount of the WSX ligand can be used in a method of immunopotentialization or to improve immune  
20 function in a mammal.

On the other hand, WSX receptor or WSX ligand antagonists (such as WSX receptor ECD or immunoadhesin, and WSX receptor or OB protein neutralizing antibodies) may be used in the treatment of those disorders wherein unacceptable lymphocyte levels are present in the mammal, particularly where this is caused by excessive activation of the WSX receptor. Examples of conditions in which administration of such an  
25 antagonist may be beneficial include: neoplastic disorders (such as Hodgkin's disease; lymphosarcoma; lymphoblastoma; lymphocytic leukemia; and lymphoma) and lymphocytosis.

Diseases or disorders in which an increase in erythropoiesis may be beneficial include, but are not limited to: erythrocytopenia; erythrodegenerative disorders; erythroblastopenia; leukoerythroblastosis; erythroclasis; thalassemia; and anemia (*e.g.* hemolytic anemia, such as acquired, autoimmune, or  
30 microangiopathic hemolytic anemia; aplastic anemia; congenital anemia, *e.g.*, congenital dyserythropoietic anemia, congenital hemolytic anemia or congenital hypoplastic anemia; dyshemopoietic anemia; Facioni's anemia; genetic anemia; hemorrhagic anemia; hyperchromic or hypochromic anemia; nutritional, hypoferric, or iron deficiency anemia; hypoplastic anemia; infectious anemia; lead anemia; local anemia; macrocytic or microcytic anemia; malignant or pernicious anemia; megaloblastic anemia; molecular anemia; normocytic anemia;  
35 physiologic anemia; traumatic or posthemorrhagic anemia; refractory anemia; radiation anemia; sickle cell anemia; splenic anemia; and toxic anemia).

Conversely, WSX receptor or WSX ligand antagonists may be used to treat those conditions in which excessive erythrocyte levels are present in a mammal, *e.g.* in neoplastic disorders such as erythroleukemia; erythroblastosis; and erythrocythemia or polycythemia.



An increase in myelopoiesis may be beneficial in any of the above-mentioned diseases or disorders as well as the following exemplary conditions: myelofibrosis; thrombocytopenia; hypoplasia; disseminated intravascular coagulation (DIC); immune (autoimmune) thrombocytopenic purpura (ITP); HIV induced ITP; myelodysplasia; thrombocytotic diseases and thrombocytosis.

5 Antagonists of the WSX receptor-WSX ligand interaction may also be used to treat myeloid cell-related conditions such as malignancies (e.g. myelosarcoma, myeloblastoma, myeloma, myeloleukemia and myelocytomatosis); myeloblastosis; myelocytosis; and myelosis.

The method may further involve the step of exposing hematopoietic cells (whether they be in cell culture or in a mammal) to one or more other cytokines (e.g. lineage-specific cytokines) and this may lead to a synergistic enhancement of the proliferation and/or differentiation of the cells. Exemplary cytokines include  
 10 thrombopoietin (TPO); erythropoietin (EPO); macrophage-colony stimulating factor (M-CSF); granulocyte-macrophage-CSF (GM-CSF); granulocyte-CSF (G-CSF); interleukin-1 (IL-1); IL-1 $\alpha$ ; IL-2; IL-3; IL-4; IL-5; IL-6; IL-7; IL-8; IL-9; IL-11; IL10; IL-12; leukemia inhibitory factor (LIF) or kit ligand (KL). In this embodiment, exposure to the cytokine may proceed, occur simultaneously with, or follow, exposure to the WSX ligand.  
 15 Preferably, the WSX ligand and one or more further cytokines are administered simultaneously to the patient (where the method is an *in vivo* one) and, optionally, are combined to form a pharmaceutical composition.

For use in the above methods, the invention also provides an article of manufacture, comprising: a container; a label on the container; and a composition comprising an active agent within the container; wherein the composition is effective for enhancing proliferation and/or differentiation of cells comprising the WSX  
 20 receptor in a mammal, the label on the container indicates that the composition can be used for enhancing proliferation and/or differentiation of those cells and the active agent in the composition is a WSX ligand. Optionally, the article of manufacture includes one or more further containers which hold further cytokine(s) in a packaged combination with the container holding the WSX ligand.

In another embodiment, an effective amount of the WSX ligand may be used to improve engraftment  
 25 in bone marrow transplantation or to stimulate mobilization of hematopoietic stem cells in a mammal prior to harvesting hematopoietic progenitors from the peripheral blood thereof.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figs. 1A-H together depict the double stranded nucleotide (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) encoding full length human WSX receptor variant 13.2. Nucleotides are numbered  
 30 at the beginning of the sense strand. Amino acid residues are numbered at the beginning of the amino acid sequence. Restriction enzyme sites are depicted above the nucleotide sequence.

Figs. 2A-B together depict an amino acid sequence alignment of full length human WSX receptor variants 6.4 (SEQ ID NO:3), 12.1 (SEQ ID NO:4) and 13.2, respectively. Homologous residues are boxed. WSX receptor variants 6.4, 12.1 and 13.2 are native sequence human WSX receptor variants which, without  
 35 being bound to any one theory, appear to be generated by alternate splicing of WSX receptor mRNA. The putative signal peptide, transmembrane, Box 1, Box 2, and Box 3 domains are indicated. The extracellular and cytoplasmic domains are amino- and carboxy-terminal, respectively, to the transmembrane domain. The Box 1-3 domains shown correspond to the box 1-3 motifs described in Baumann *et al.*, *Mol. Cell. Biol.* 14(1):138-146 (1994).

Figs. 3A-E together depict an alignment of the nucleotide sequences encoding human WSX receptor variants 6.4 (SEQ ID NO:5), 12.1 (SEQ ID NO:6) and 13.2, respectively.

Figs. 4A-B depict an alignment of the full length human WSX receptor variant 13.2 amino acid sequence (top) with that of partial murine WSX receptor extracellular domain sequence (bottom) (SEQ ID NO:7) obtained as described in Example 7. The putative murine signal peptide is marked with an arrow.

Figs. 5A-F represent an alignment of the nucleotide sequences encoding human WSX receptor variant 13.2 (bottom) and partial murine WSX receptor extracellular domain (top) (SEQ ID NO:8), respectively.

Fig. 6 is a bar graph depicting results of the thymidine incorporation assay described in Example 5. <sup>3</sup>H-thymidine incorporation (counts per minute, CPM) in parental Baf3 cells or Baf3 cells electroporated with GH/WSX variant 13.2 chimera in the presence of varying concentrations of human growth hormone (GH) is shown.

Fig. 7 shows the human and murine oligonucleotides (SEQ ID NOS:9-38, respectively) used for the antisense experiment described in Example 8.

Figs. 8 and 9 show thymidine incorporation assays in Baf-3 cells. For these assays, cells were deprived of IL-3 for 16-18 hours (in RPMI 1640 supplemented with 10% fetal calf serum (FCS)). Cells were washed in serum free RPMI 1640 and plated at 50,000 cells per well in 0.2 mls of serum free RPMI 1640 supplemented with the indicated concentration of human GH or human OB protein. Cells were stimulated for 24 hours and thymidine incorporation was determined as described (Zeigler *et al. Blood* 84:2422-2430 (1994)). Assays were performed in triplicate and the results were confirmed in three independent experiments.

In Fig. 8, GH receptor-WSX receptor variant 12.1 or 13.2 chimeric proteins were expressed in Baf-3 cells as described in Example 5. These transfected cells and the parental Baf-3 line were stimulated with hGH and the incorporation of titrated thymidine determined.

In Fig. 9, Baf-3 cells were stably transfected with WSX receptor variant 13.2. Thymidine incorporation was then determined in these cell lines following stimulation with human OB protein.

In Figs. 10A-C, murine fetal liver AA4<sup>+</sup>Sca<sup>+</sup>Kit<sup>+</sup> (fASK) stem cells were cultured in suspension culture or methylcellulose. In Fig. 10A, fASK cells were cultured in suspension culture containing serum with kit ligand (KL) or kit ligand and OB protein. Cell counts and cytopsin analyses were performed 7 days later. In Fig. 10B, fASK cells were seeded into methylcellulose under either myeloid or lymphoid conditions as described in Example 10. Colony counts were performed 14 days later. For colonies produced under lymphoid conditions, FACS analysis demonstrated the vast majority of cells to be B220 positive. In Fig. 10C, fASK cells were seeded into methylcellulose containing kit ligand. To this base media, erythropoietin (EPO) or erythropoietin and OB protein were then added. The resultant colonies were counted 14 days later. FACS analysis demonstrated approximately 95% of these colonies to be TER 119 positive. All assays were performed in triplicate and confirmed in at least three independent experiments.

Fig. 11 illustrates methylcellulose assays to determine the colony forming potential of *db/db*, *ob/ob* and the corresponding wild-type marrow. 100,000 bone marrow cells were seeded into methylcellulose and the resultant colonies counted after 14 days. Assays were performed using both myeloid and lymphoid conditions. Assays were performed in triplicate and the experiments were repeated a minimum of 3 times.

Figs. 12A-B show bone marrow cellular profiles in wild-type misty gray homozygotes, misty gray/*db* heterozygotes, and homozygote *db/db* mice. Overall cellularity in the *db/db* marrow was unchanged compared to controls. Fig. 12A shows cellular profiles determined using anti-B220, anti-CD43, and anti-TER119 antibodies. Fig. 12B shows cellular profiles of the spleens from the above groups.

5 Figs. 13A-C are an analysis of peripheral blood in *db/db* homozygotes, *db/db* misty gray heterozygotes and misty gray homozygotes. 40 microliters of peripheral blood was taken via orbital bleed and analyzed on a Serrono Baker system 9018. All areas described by the boxes represent the mean  $\pm$  one standard deviation of the two parameters.

10 Fig. 14 is a comparison of peripheral lymphocyte counts and blood glucose level. Five groups of animals, misty-gray, misty-gray/*db*, *db/db*, interferon  $\alpha$ -transgenic, and glucokinase transgenic heterozygote mice (gLKa) were sampled via retro-orbital bleed. Blood glucose levels in these mice were determined. All areas described by the boxes represent the mean  $\pm$  standard deviation of the two parameters.

15 In Figs. 15A-C, misty gray homozygotes, *db*/misty gray heterozygotes, and homozygous *db/db* mice were subjected to sub-lethal irradiation and the recovery kinetics of the peripheral blood was determined via retro-orbital bleeds.

Figs. 16A-16Q together show the nucleotide sequence (SEQ ID NO:46) and the amino acid sequence (SEQ ID NO: 47) of the human OB-immunoglobulin chimera in the plasmid described in of Example 11.

20 Fig. 17 shows binding of anti-WSX receptor agonist antibodies to human WSX receptor. The anti-WSX receptor agonist antibodies (2D7 and 1G4) produced as described in Example 13 and an IgG isotope control were evaluated for their ability to bind to human WSX receptor by capture ELISA.

Fig. 18 shows the activity of mAbs 2D7 and 1G4 as well as OB protein in the KIRA ELISA (see Example 13). Absorbance at 490nm versus concentration of antibody or ligand in this assay is shown.

25 Fig. 19 depicts binding of anti-WSX receptor agonist antibodies to murine WSX receptor. The anti-WSX receptor agonist antibodies (2D7 and 1G4) and an IgG isotope control were evaluated for their ability to bind to murine WSX receptor by capture ELISA.

30 Figs. 20A-B show the results of epitope mapping of the agonist anti-WSX receptor antibodies produced as described in Example 13. Fig. 20A shows blocking ability of anti-WSX receptor antibodies on Epitope A using biotinylated 2D7. Fig. 20B shows blocking ability of anti-WSX receptor antibodies on Epitope B using biotinylated 1C11. Based on the competitive binding ELISA, 2D7 bound a different epitope from 1E11, 1C11 and 1G4.

Fig. 21 depicts an alignment of the amino acid sequences of full length human WSX receptor variant 6.4 (hWSXR) (SEQ ID NO:3) and murine WSX receptor (mWSXR) (SEQ ID NO:51).

Fig. 22 is a standard curve for human OB protein in the KIRA ELISA, which illustrates schematically inside the graph WSX receptor KIRA ELISA panning with scFv phage as described in Example 14.

35 Fig. 23 shows the activity of clone # 3, #4 and # 17 scFv phage from Example 14 and anti-HER2 scFv phage control in the KIRA ELISA. Absorbance versus phage titer is shown.

Fig. 24 shows the activity of clone # 3, #4 and # 17 scFv from Example 14, anti-HER2 scFv control (Her2 clone) and OB protein in the KIRA ELISA. Absorbance versus antibody concentration is shown.

Fig. 25 aligns the amino acid sequences of agonist antibody clone #3 (3.scFv) (SEQ ID NO:48), clone #4 (4.scFv) (SEQ ID NO:49) and clone #17 (17.scFv) (SEQ ID NO:50) obtained as described in Example 14. Complementarity determining region (CDR) residues according to Kabat *et al.*, *Sequences of Proteins of Immunological Interest*. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991) are underlined and hypervariable loop residues (Chothia *et al.*, *Nature* 342:8767 (1989)) are in italics.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

### I. Definitions

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

The terms "WSX receptor" or "WSX receptor polypeptide" when used herein encompass native sequence WSX receptor; WSX receptor variants; WSX extracellular domain; and chimeric WSX receptor (each of which is defined herein). Optionally, the WSX receptor is not associated with native glycosylation. "Native glycosylation" refers to the carbohydrate moieties which are covalently attached to WSX receptor when it is produced in the mammalian cell from which it is derived in nature. Accordingly, human WSX receptor produced in a non-human cell is an example of a WSX receptor which is "not associated with native glycosylation". Sometimes, the WSX receptor is unglycosylated (*e.g.*, as a result of being produced recombinantly in a prokaryote).

"WSX ligand" is a molecule which binds to and activates native sequence WSX receptor (especially WSX receptor variant 13.2). The ability of a molecule to bind to WSX receptor can be determined by the ability of a putative WSX ligand to bind to WSX receptor immunoadhesin (see Example 2) coated on an assay plate, for example. The thymidine incorporation assay provides a means for screening for WSX ligands which activate the WSX receptor. Exemplary WSX ligands include anti-WSX receptor agonist antibodies and OB protein (*e.g.*, described in Zhang *et al.* *Nature* 372:425-431 (1994)).

The terms "OB protein" and "OB" are used interchangeably herein and refer to native sequence OB proteins (also known as "leptins") and their functional derivatives.

A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide (*e.g.*, WSX receptor or OB protein) derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino acid sequence of naturally occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

The term "native sequence WSX receptor" specifically encompasses naturally-occurring truncated forms of the WSX receptor, naturally-occurring variant forms (*e.g.*, alternatively spliced forms such as human WSX receptor variants 6.4, 12.1 and 13.2 described herein) and naturally-occurring allelic variants of the WSX receptor. The preferred native sequence WSX receptor is a mature native sequence human WSX receptor, such as human WSX receptor variant 6.4, human WSX receptor variant 12.1 or human WSX receptor variant 13.2 (each shown in Figs. 2A-B). Most preferred is mature human WSX receptor variant 13.2.

The term "native sequence OB protein" includes those OB proteins from any animal species (*e.g.* human, murine, rabbit, cat, cow, sheep, chicken, porcine, equine, etc.) as occurring in nature. The definition specifically includes variants with or without a glutamine at amino acid position 49, using the amino acid

numbering of Zhang *et al.*, *supra*. The term "native sequence OB protein" includes the native proteins with or without the initiating N-terminal methionine (Met), and with or without the native signal sequence, either in monomeric or in dimeric form. The native sequence human and murine OB proteins known in the art are 167 amino acids long, contain two conserved cysteines, and have the features of a secreted protein. The protein is largely hydrophilic, and the predicted signal sequence cleavage site is at position 21, using the amino acid numbering of Zhang *et al.*, *supra*. The overall sequence homology of the human and murine sequences is about 84%. The two proteins show a more extensive identity in the N-terminal region of the mature protein, with only four conservative and three non-conservative substitutions among the residues between the signal sequence cleavage site and the conserved Cys at position 117. The molecular weight of OB protein is about 16 kD in a monomeric form.

The "WSX receptor extracellular domain" (ECD) is a form of the WSX receptor which is essentially free of the transmembrane and cytoplasmic domains of WSX receptor, *i.e.*, has less than 1% of such domains, preferably 0.5 to 0% of such domains, and more preferably 0.1 to 0% of such domains. Ordinarily, the WSX receptor ECD will have an amino acid sequence having at least about 95% amino acid sequence identity with the amino acid sequence of the ECD of WSX receptor indicated in Figs. 2A-B for human WSX receptor variants 6.4, 12.1 and 13.2, preferably at least about 98%, more preferably at least about 99% amino acid sequence identity, and thus includes WSX receptor variants as defined below.

A "variant" polypeptide means a biologically active polypeptide as defined below having less than 100% sequence identity with a native sequence polypeptide (*e.g.*, WSX receptor having the deduced amino acid sequence shown in Figs. 1A-H for human WSX receptor variant 13.2). Such variants include polypeptides wherein one or more amino acid residues are added at the N- or C-terminus of, or within, the native sequence; from about one to thirty amino acid residues are deleted, and optionally substituted by one or more amino acid residues; and derivatives of the above polypeptides, wherein an amino acid residue has been covalently modified so that the resulting product has a non-naturally occurring amino acid. Ordinarily, a biologically active WSX receptor variant will have an amino acid sequence having at least about 90% amino acid sequence identity with human WSX receptor variant 13.2 shown in Figs. 1A-H, preferably at least about 95%, more preferably at least about 99%. Ordinarily, a biologically active OB protein variant will have an amino acid sequence having at least about 90% amino acid sequence identity with a native sequence OB protein, preferably at least about 95%, more preferably at least about 99%.

A "chimeric" OB protein or WSX receptor is a polypeptide comprising OB protein or full-length WSX receptor or one or more domains thereof (*e.g.*, the extracellular domain of the WSX receptor) fused or bonded to heterologous polypeptide. The chimeric WSX receptor will generally share at least one biological property in common with human WSX receptor variant 13.2. The chimeric OB protein will generally share at least one biological property in common with a native sequence OB protein. Examples of chimeric polypeptides include immunoadhesins and epitope tagged polypeptides.

The term "WSX immunoadhesin" is used interchangeably with the expression "WSX receptor-immunoglobulin chimera" and refers to a chimeric molecule that combines a portion of the WSX receptor (generally the extracellular domain thereof) with an immunoglobulin sequence. Likewise, an "OB protein immunoadhesin" or "OB-immunoglobulin chimera" refers to a chimeric molecule which combines OB protein

(or a portion thereof) with an immunoglobulin sequence. The immunoglobulin sequence preferably, but not necessarily, is an immunoglobulin constant domain. The immunoglobulin moiety in the chimeras of the present invention may be obtained from IgG1, IgG2, IgG3 or IgG4 subtypes, IgA, IgE, IgD or IgM, but preferably IgG1 or IgG3.

5           The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising WSX receptor or OB protein fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody thereagainst can be made, yet is short enough such that it does not interfere with biological activity of the WSX receptor or OB protein. The tag polypeptide preferably also is fairly unique so that the antibody thereagainst does not substantially cross-react with other epitopes. Suitable tag polypeptides  
10           generally have at least six amino acid residues and usually between about 8-50 amino acid residues (preferably between about 9-30 residues).

          "Isolated" WSX receptor (or OB protein) means WSX receptor (or OB protein) that has been purified from a WSX receptor (or OB protein) source or has been prepared by recombinant or synthetic methods and is sufficiently free of other peptides or proteins (1) to obtain at least 15 and preferably 20 amino acid residues of  
15           the N-terminal or of an internal amino acid sequence by using a spinning cup sequenator or the best commercially available amino acid sequenator marketed or as modified by published methods as of the filing date of this application, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Homogeneity here means less than about 5% contamination with other source proteins.

20           "Essentially pure" protein means a composition comprising at least about 90% by weight of the protein, based on total weight of the composition, preferably at least about 95% by weight. "Essentially homogeneous" protein means a composition comprising at least about 99% by weight of protein, based on total weight of the composition.

          "Biological property" when used in conjunction with either "WSX receptor" or "isolated WSX receptor"  
25           means having an effector or antigenic function or activity that is directly or indirectly caused or performed by native sequence WSX receptor (whether in its native or denatured conformation). Effector functions include ligand binding; and enhancement of survival, differentiation and/or proliferation of cells (especially proliferation of cells). However, effector functions do not include possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against native sequence WSX receptor.

30           "Biological property" when used in conjunction with either "OB protein" or "isolated OB protein" means having an effector function that is directly or indirectly caused or performed by native sequence OB protein. Effector functions of native sequence OB protein include WSX receptor binding and activation; and enhancement of differentiation and/or proliferation of cells expressing this receptor (as determined in the thymidine incorporation assay, for example). A "biologically active" OB protein is one which possesses a  
35           biological property of native sequence OB protein.

          A "functional derivative" of a native sequence OB protein is a compound having a qualitative biological property in common with a native sequence OB protein. "Functional derivatives" include, but are not limited to, fragments of native sequence OB proteins and derivatives of native sequence OB proteins and their fragments, provided that they have a biological activity in common with a corresponding native sequence OB protein. The

term "derivative" encompasses both amino acid sequence variants of OB protein and covalent modifications thereof.

The phrase "long half-life" as used in connection with OB derivatives, concerns OB derivatives having a longer plasma half-life and/or slower clearance than a corresponding native sequence OB protein. The long half-life derivatives preferably will have a half-life at least about 1.5-times longer than a native OB protein; more preferably at least about 2-times longer than a native OB protein, more preferably at least about 3-time longer than a native OB protein. The native OB protein preferably is that of the individual to be treated.

An "antigenic function" means possession of an epitope or antigenic site that is capable of cross-reacting with antibodies raised against native sequence WSX receptor. The principal antigenic function of a WSX receptor is that it binds with an affinity of at least about  $10^6$  L/mole to an antibody raised against native sequence WSX receptor. Ordinarily, the polypeptide binds with an affinity of at least about  $10^7$  L/mole. The antibodies used to define "antigenic function" are rabbit polyclonal antibodies raised by formulating the WSX receptor in Freund's complete adjuvant, subcutaneously injecting the formulation, and boosting the immune response by intraperitoneal injection of the formulation until the titer of the anti-WSX receptor or antibody plateaus.

"Biologically active" when used in conjunction with either "WSX receptor" or "isolated WSX receptor" means a WSX receptor polypeptide that exhibits or shares an effector function of native sequence WSX receptor and that may (but need not) in addition possess an antigenic function. A principal effector function of the WSX receptor is its ability to induce proliferation of CD34+ human umbilical cord blood cells in the colony assay described in Example 8.

"Antigenically active" WSX receptor is defined as a polypeptide that possesses an antigenic function of WSX receptor and that may (but need not) in addition possess an effector function.

"Percent amino acid sequence identity" is defined herein as the percentage of amino acid residues in the candidate sequence that are identical with the residues in the native sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. None of N-terminal, C-terminal, or internal extensions, deletions, or insertions into the candidate sequence shall be construed as affecting sequence identity or homology.

A "thymidine incorporation assay" can be used to screen for molecules which activate the WSX receptor. In order to perform this assay, IL-3 dependent Baf3 cells (Palacios *et al.*, *Cell*, 41:727-734 (1985)) are stably transfected with full length native sequence WSX receptor as described in Example 4. The WSX receptor/Baf3 cells so generated are starved of IL-3 for, e.g., 24 hours in a humidified incubator at 37°C in 5%CO<sub>2</sub> and air. Following IL-3 starvation, the cells are plated out in 96 well culture dishes with, or without, a test sample containing a potential agonist (such test samples are optionally diluted) and cultured for 24 hours in a cell culture incubator. 20μl of serum free RPMI media containing 1μCi of <sup>3</sup>H thymidine is added to each well for the last 6-8 hours. The cells are then harvested in 96 well filter plates and washed with water. The filters are then counted using a Packard Top Count Microplate Scintillation Counter, for example. Agonists are expected to induce a statistically significant increase (to a P value of 0.05) in <sup>3</sup>H uptake, relative to control. Preferred agonists leads to an increase in <sup>3</sup>H uptake which is at least two fold of that of the control.

An "isolated" WSX receptor nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the WSX receptor nucleic acid. An isolated WSX receptor nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated WSX receptor nucleic acid molecules therefore are distinguished from the WSX receptor nucleic acid molecule as it exists in natural cells. However, an isolated WSX receptor nucleic acid molecule includes WSX receptor nucleic acid molecules contained in cells that ordinarily express WSX receptor where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The expression "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and possibly, other as yet poorly understood sequences. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

The term "antibody" is used in the broadest sense and specifically covers monoclonal antibodies, antibody compositions with polyepitopic specificity, bispecific antibodies, diabodies, and single-chain molecules, as well as antibody fragments (*e.g.*, Fab, F(ab')<sub>2</sub>, and Fv), so long as they exhibit the desired biological activity.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the



character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature* 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567 (Cabilly *et al.*)). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (Cabilly *et al.*, *supra*; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)).

"Humanized" forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Reichmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). The humanized antibody includes a Primatized<sup>TM</sup> antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest.

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*i.e.* residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*i.e.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the

heavy chain variable domain; Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

"Non-immunogenic in a human" means that upon contacting the polypeptide of interest in a physiologically acceptable carrier and in a therapeutically effective amount with the appropriate tissue of a human, no state of sensitivity or resistance to the polypeptide of interest is demonstrable upon the second administration of the polypeptide of interest after an appropriate latent period (*e.g.*, 8 to 14 days).

By "agonist antibody" is meant an antibody which is able to activate native sequence WSX receptor. The agonist antibody of particular interest herein is one which mimics one or more (*e.g.* all) of the biological properties of naturally occurring WSX ligand, OB protein. In preferred embodiments, the agonist antibody has a quantitative biological property of OB protein which is within about two orders of magnitude, and preferably within about one order of magnitude, that of OB protein. The agonist antibody may bind to and activate WSX receptor and thereby stimulate proliferation and/or differentiation and/or maturation and/or survival of a cell which expresses the WSX receptor (*e.g.* WSX receptor variant 13.2). In this embodiment of the invention, the agonist antibody may be one which enhances proliferation and/or differentiation of a hematopoietic progenitor cell which expresses the WSX receptor at its cell surface; enhances proliferation and/or differentiation of lymphoid blood cell lineages; enhances proliferation and/or differentiation of myeloid blood cell lineages; and/or enhances proliferation and/or differentiation of erythroid blood cell lineages. The agonist antibody may display agonist activity upon binding to a chimeric receptor comprising the WSX receptor extracellular domain in the KIRA ELISA. The agonist antibody may stimulate  $^3\text{H}$  uptake in the thymidine incorporation assay using a signaling WSX receptor (see above); decrease body weight and/or fat-depot weight and/or food intake in an obese mammal (*e.g.* in the *ob/ob* mouse); effect  $\text{Ca}^{2+}$  influx in adipocytes; and/or activate downstream signaling molecules of OB protein.

A "neutralizing antibody" is one which is able to block or significantly reduce an effector function of native sequence WSX receptor or OB protein. For example, a neutralizing antibody may inhibit or reduce WSX receptor activation by a WSX ligand as determined in the thymidine incorporation assay or in a KIRA ELISA.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.*,  $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{90}\text{Y}$  and  $^{186}\text{Re}$ ), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include Adriamycin, Doxorubicin, 5-Fluorouracil, Cytosine arabinoside ("Ara-C"), Cyclophosphamide, Thiotepa, Taxotere (docetaxel), Busulfan, Cytosine, Taxol, Methotrexate, Cisplatin, Melphalan, Vinblastine, Bleomycin, Etoposide, Ifosfamide, Mitomycin C, Mitoxantrone, Vincristine, Vinorelbine, Carboplatin, Teniposide, Daunomycin, Carminomycin, Aminopterin, Dactinomycin, Mitomycins, Esperamicins (see U.S. Pat. No. 4,675,187), Melphalan and other related nitrogen mustards.

The term "prodrug" as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, *e.g.*, Wilman, "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions*, 14, pp. 375-382, 615th Meeting Belfast (1986).

and Stella *et al.*, "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery*, Borchardt *et al.*, (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs,  $\beta$ -lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

10 An "antagonist" of the WSX receptor and/or OB protein is a molecule which prevents, or interferes with, binding and/or activation of the WSX receptor or OB protein. Such molecules can be screened for their ability to competitively inhibit WSX receptor activation by OB protein in the thymidine incorporation assay disclosed herein, for example. Examples of such molecules include: WSX receptor ECD; WSX receptor immunoadhesin; neutralizing antibodies against WSX receptor or OB protein; small molecule and peptide  
15 antagonists; and antisense nucleotides against the WSX receptor or *ob* gene.

The phrase "enhancing proliferation of a cell" encompasses the step of increasing the extent of growth and/or reproduction of the cell relative to an untreated cell either *in vitro* or *in vivo*. An increase in cell proliferation in cell culture can be detected by counting the number of cells before and after exposure to a molecule of interest. The extent of proliferation can be quantified via microscopic examination of the degree  
20 of confluency. Cell proliferation can also be quantified using the thymidine incorporation assay described herein.

By "enhancing differentiation of a cell" is meant the act of increasing the extent of the acquisition or possession of one or more characteristics or functions which differ from that of the original cell (*i.e.* cell specialization). This can be detected by screening for a change in the phenotype of the cell (*e.g.*, identifying morphological changes in the cell).

25 A "hematopoietic progenitor cell" or "primitive hematopoietic cell" is one which is able to differentiate to form a more committed or mature blood cell type.

"Lymphoid blood cell lineages" are those hematopoietic precursor cells which are able to differentiate to form lymphocytes (B-cells or T-cells). Likewise, "lymphopoiesis" is the formation of lymphocytes.

"Erythroid blood cell lineages" are those hematopoietic precursor cells which are able to differentiate  
30 to form erythrocytes (red blood cells) and "erythropoiesis" is the formation of erythrocytes.

The phrase "myeloid blood cell lineages", for the purposes herein, encompasses all hematopoietic precursor cells, other than lymphoid and erythroid blood cell lineages as defined above, and "myelopoiesis" involves the formation of blood cells (other than lymphocytes and erythrocytes).

A "CD34+ cell population" is enriched for hematopoietic stem cells. A CD34+ cell population can be  
35 obtained from umbilical cord blood or bone marrow, for example. Human umbilical cord blood CD34+ cells can be selected for using immunomagnetic beads sold by Miltenyi (California), following the manufacturer's directions.

"Physiologically acceptable" carriers, excipients, or stabilizers are ones which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically

acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronic or polyethylene glycol (PEG).

As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG1, IgG2, IgG3, and IgG4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule. Exemplary salvage receptor binding epitope sequences include HQNLSDGK (SEQ ID NO:39); HQNISDGK (SEQ ID NO:40); HQSLGTQ (SEQ ID NO:41); VISSLGQ (SEQ ID NO:42); and PKNSSMISNTP (SEQ ID NO:43).

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are OB protein; growth hormones such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- $\alpha$  and - $\beta$ ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- $\beta$ ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- $\alpha$  and TGF- $\beta$ ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- $\alpha$ , - $\beta$ , and - $\gamma$ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 $\alpha$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; and other polypeptide factors including leukemia inhibitory factor (LIF) and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

A "lineage-specific cytokine" is one which acts on relatively committed cells in the hematopoietic cascade and gives rise to an expansion in blood cells of a single lineage. Examples of such cytokines include EPO, TPO, and G-CSF.

"Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented.

The term "obesity" is used to designate a condition of being overweight associated with excessive bodily fat. The desirable weight for a certain individual depends on a number of factors including sex, height, age, overall built, etc. The same factors will determine when an individual is considered obese. The determination of an optimum body weight for a given individual is well within the skill of an ordinary physician.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, *etc.* Preferably, the mammal is human.

By "solid phase" is meant a non-aqueous matrix to which a reagent of interest (*e.g.* the WSX receptor or an antibody thereto) can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.* controlled pore glass), polysaccharides (*e.g.* agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (*e.g.* an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

## II. Modes for Carrying Out the Invention

The present invention is based on the discovery of the WSX receptor. The experiments described herein demonstrate that this molecule is a cytokine receptor which appears to play a role in enhancing proliferation and/or differentiation of hematopoietic cells. In particular, this receptor has been found to be present in enriched human stem cell populations, thus indicating that WSX ligands, such as agonist antibodies, may be used to stimulate proliferation of hematopoietic stem cells/progenitor cells. Other uses for this receptor will be apparent from the following discussion. A description follows as to how WSX receptor or OB proteins may be prepared.

### a. Preparation of WSX Receptor or OB Protein

Techniques suitable for the production of WSX receptor or OB protein are well known in the art and include isolating WSX receptor or OB protein from an endogenous source of the polypeptide, peptide synthesis (using a peptide synthesizer) and recombinant techniques (or any combination of these techniques). The preferred technique for production of WSX receptor or OB protein is a recombinant technique to be described below.

Most of the discussion below pertains to recombinant production of WSX receptor or OB protein by culturing cells transformed with a vector containing WSX receptor or OB protein nucleic acid and recovering the polypeptide from the cell culture. It is further envisioned that the WSX receptor or OB protein of this invention may be produced by homologous recombination, as provided for in WO 91/06667, published 16 May 1991.

Briefly, this method involves transforming primary human cells containing a WSX receptor or OB protein-encoding gene with a construct (*i.e.*, vector) comprising an amplifiable gene (such as dihydrofolate reductase (DHFR) or others discussed below) and at least one flanking region of a length of at least about 150 bp that is homologous with a DNA sequence at the locus of the coding region of the WSX receptor or OB protein gene to provide amplification of the WSX receptor or OB protein gene. The amplifiable gene must be at a site that does not interfere with expression of the WSX receptor or OB protein gene. The transformation is conducted such that the construct becomes homologously integrated into the genome of the primary cells to define an amplifiable region.

Primary cells comprising the construct are then selected for by means of the amplifiable gene or other marker present in the construct. The presence of the marker gene establishes the presence and integration of the

construct into the host genome. No further selection of the primary cells need be made, since selection will be made in the second host. If desired, the occurrence of the homologous recombination event can be determined by employing PCR and either sequencing the resulting amplified DNA sequences or determining the appropriate length of the PCR fragment when DNA from correct homologous integrants is present and expanding only those cells containing such fragments. Also if desired, the selected cells may be amplified at this point by stressing the cells with the appropriate amplifying agent (such as methotrexate if the amplifiable gene is DHFR), so that multiple copies of the target gene are obtained. Preferably, however, the amplification step is not conducted until after the second transformation described below.

After the selection step, DNA portions of the genome, sufficiently large to include the entire amplifiable region, are isolated from the selected primary cells. Secondary mammalian expression host cells are then transformed with these genomic DNA portions and cloned, and clones are selected that contain the amplifiable region. The amplifiable region is then amplified by means of an amplifying agent if not already amplified in the primary cells. Finally, the secondary expression host cells now comprising multiple copies of the amplifiable region containing WSX receptor or OB protein are grown so as to express the gene and produce the protein.

**i. Isolation of DNA Encoding WSX Receptor or OB Protein**

The DNA encoding WSX receptor or OB protein may be obtained from any cDNA library prepared from tissue believed to possess the WSX receptor or OB protein mRNA and to express it at a detectable level. Accordingly, WSX receptor or OB protein DNA can be conveniently obtained from a cDNA library prepared from mammalian fetal liver. The WSX receptor or OB protein-encoding gene may also be obtained from a genomic library or by oligonucleotide synthesis.

Libraries are screened with probes (such as antibodies to the WSX receptor or OB protein, or oligonucleotides of about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding WSX receptor or OB protein is to use PCR methodology as described in section 14 of Sambrook *et al.*, *supra*.

A preferred method of practicing this invention is to use carefully selected oligonucleotide sequences to screen cDNA libraries from various human tissues, preferably human fetal liver. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized.

The oligonucleotide must be labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of labeling is to use <sup>32</sup>P-labeled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

Amino acid sequence variants of WSX receptor or OB protein are prepared by introducing appropriate nucleotide changes into the WSX receptor or OB protein DNA, or by synthesis of the desired WSX receptor or OB protein. Such variants represent insertions, substitutions, and/or specified deletions of, residues within or at one or both of the ends of the amino acid sequence of a naturally occurring human WSX receptor or OB protein, such as the WSX receptor variants shown in Figs 2A-B or the human OB protein of Zhang *et al.*, *supra*.

Preferably, these variants represent insertions and/or substitutions within or at one or both ends of the mature sequence, and/or insertions, substitutions and/or specified deletions within or at one or both of the ends of the signal sequence of the WSX receptor or OB protein. Any combination of insertion, substitution, and/or specified deletion is made to arrive at the final construct, provided that the final construct possesses the desired biological activity as defined herein. The amino acid changes also may alter post-translational processes of the WSX receptor or OB protein, such as changing the number or position of glycosylation sites, altering the membrane anchoring characteristics, and/or altering the intracellular location of the WSX receptor or OB protein by inserting, deleting, or otherwise affecting the leader sequence of the WSX receptor or OB protein.

Variations in the native sequence as described above can be made using any of the techniques and guidelines for conservative and non-conservative mutations set forth in U.S. Pat. No. 5,364,934. These include oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. See also, for example, Table I therein and the discussion surrounding this table for guidance on selecting amino acids to change, add, or delete.

## ii. Insertion of Nucleic Acid into Replicable Vector

The nucleic acid (*e.g.*, cDNA or genomic DNA) encoding the WSX receptor or OB protein is inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

### (1) Signal sequence component

The WSX receptor or OB proteins of this invention may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the WSX receptor or OB protein DNA that is inserted into the vector. The heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native WSX receptor or OB protein signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, *e.g.*, the yeast invertase leader,  $\alpha$  factor leader (including *Saccharomyces* and *Kluyveromyces*  $\alpha$ -factor leaders, the latter described in U.S. Pat. No. 5,010,182 issued 23 April 1991), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression the native signal sequence (*e.g.*, the WSX receptor or OB protein presequence that normally directs secretion of WSX receptor or OB protein from human cells *in vivo*) is satisfactory, although other mammalian signal sequences may be suitable, such as signal sequences from other animal WSX receptors or OB proteins, and signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders, for example, the herpes simplex gD signal.

The DNA for such precursor region is ligated in reading frame to DNA encoding the mature WSX receptor or OB protein.

(2) *Origin of replication component*

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 $\mu$  plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

Most expression vectors are "shuttle" vectors, *i.e.*, they are capable of replication in at least one class of organisms but can be transfected into another organism for expression. For example, a vector is cloned in *E. coli* and then the same vector is transfected into yeast or mammalian cells for expression even though it is not capable of replicating independently of the host cell chromosome.

DNA may also be amplified by insertion into the host genome. This is readily accomplished using *Bacillus* species as hosts, for example, by including in the vector a DNA sequence that is complementary to a sequence found in *Bacillus* genomic DNA. Transfection of *Bacillus* with this vector results in homologous recombination with the genome and insertion of WSX receptor or OB protein DNA. However, the recovery of genomic DNA encoding WSX receptor or OB protein is more complex than that of an exogenously replicated vector because restriction enzyme digestion is required to excise the WSX receptor or OB protein DNA.

(3) *Selection gene component*

Expression and cloning vectors should contain a selection gene, also termed a selectable marker. This gene encodes a protein necessary for the survival or growth of transformed host cells grown in a selective culture medium. Host cells not transformed with the vector containing the selection gene will not survive in the culture medium. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the WSX receptor or OB protein nucleic acid, such as DHFR or thymidine kinase. The mammalian cell transformants are placed under selection pressure that only the transformants are uniquely adapted to survive by virtue of having taken up the marker. Selection pressure is imposed by culturing the transformants under conditions in which the concentration of selection agent in the medium is successively changed, thereby leading to amplification of both the selection gene and the DNA that encodes WSX receptor or OB protein. Amplification is the process by which genes in greater demand for the production of a protein critical for growth are reiterated in tandem within the chromosomes of successive generations of recombinant cells. Increased quantities of WSX receptor or OB protein are synthesized from the



amplified DNA. Other examples of amplifiable genes include metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, *etc.*

For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980). The transformed cells are then exposed to increased levels of methotrexate. This leads to the synthesis of multiple copies of the DHFR gene, and, concomitantly, multiple copies of other DNA comprising the expression vectors, such as the DNA encoding WSX receptor or OB protein. This amplification technique can be used with any otherwise suitable host, *e.g.*, ATCC No. CCL61 CHO-K1, notwithstanding the presence of endogenous DHFR if, for example, a mutant DHFR gene that is highly resistant to Mtx is employed (EP 117,060).

Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding WSX receptor or OB protein, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, *e.g.*, kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 (Stinchcomb *et al.*, *Nature* 282:39 (1979)). The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, *Genetics* 85:12 (1977). The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu2*-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu2* gene.

In addition, vectors derived from the 1.6  $\mu$ m circular plasmid pKD1 can be used for transformation of *Kluyveromyces* yeasts. Bianchi *et al.*, *Curr. Genet.* 12:185 (1987). More recently, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis*. Van den Berg, *Bio/Technology* 8:135 (1990). Stable multi-copy expression vectors for secretion of mature recombinant human serum albumin by industrial strains of *Kluyveromyces* have also been disclosed. Fleer *et al.*, *Bio/Technology* 9:968-975 (1991).

#### (4) Promoter Component

Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the WSX receptor or OB protein nucleic acid. Promoters are untranslated sequences located upstream (5') to the start codon of a structural gene (generally within about 100 to 1000 bp) that control the transcription and translation of particular nucleic acid sequence, such as the WSX receptor or OB protein nucleic acid sequence, to which they are operably linked. Such promoters typically fall into two classes, inducible and constitutive. Inducible promoters are promoters that initiate increased levels of transcription from DNA under their control in response to some change in culture conditions, *e.g.*, the presence or absence of a nutrient or a change in temperature. At this time a large number of promoters recognized by a variety of potential host cells are well known. These promoters are operably linked to WSX receptor or OB protein-encoding DNA by removing the promoter from the source DNA by restriction enzyme digestion and inserting the isolated promoter

sequence into the vector. Both the native WSX receptor or OB protein promoter sequence and many heterologous promoters may be used to direct amplification and/or expression of the WSX receptor or OB protein DNA. However, heterologous promoters are preferred, as they generally permit greater transcription and higher yields of WSX receptor or OB protein as compared to the native WSX receptor or OB protein promoter.

5 Promoters suitable for use with prokaryotic hosts include the  $\beta$ -lactamase and lactose promoter systems (Chang *et al.*, *Nature* 275:615 (1978); Goeddel *et al.*, *Nature* 281:544 (1979)), alkaline phosphatase, a tryptophan (trp) promoter system (Goeddel, *Nucleic Acids Res.* 8:4057 (1980); EP 36,776), and hybrid promoters such as the tac promoter. deBoer *et al.*, *Proc. Natl. Acad. Sci. USA* 80:21-25 (1983). However, other known bacterial promoters are suitable. Their nucleotide sequences have been published, thereby enabling a skilled  
10 worker operably to ligate them to DNA encoding WSX receptor or OB protein (Siebenlist *et al.*, *Cell* 20:269 (1980)) using linkers or adaptors to supply any required restriction sites. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding WSX receptor or OB protein.

Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region  
15 located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CXCAAT region where X may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

20 Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase (Hitzeman *et al.*, *J. Biol. Chem.* 255:2073 (1980)) or other glycolytic enzymes (Hess *et al.*, *J. Adv. Enzyme Reg.* 7:149 (1968); Holland, *Biochemistry* 17:4900 (1978)), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose  
25 isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and  
30 promoters for use in yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

WSX receptor or OB protein transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma  
35 virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, from heat-shock promoters, and from the promoter normally associated with the WSX receptor or OB protein sequence, provided such promoters are compatible with the host cell systems.

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Construction of suitable vectors containing one or more of the above-listed components employs standard ligation techniques. Isolated plasmids or DNA fragments are cleaved, tailored, and re-ligated in the form desired to generate the plasmids required.

For analysis to confirm correct sequences in plasmids constructed, the ligation mixtures are used to transform *E. coli* K12 strain 294 (ATCC 31,446) and successful transformants selected by ampicillin or tetracycline resistance where appropriate. Plasmids from the transformants are prepared, analyzed by restriction endonuclease digestion, and/or sequenced by the method of Messing *et al.*, *Nucleic Acids Res.* 9:309 (1981) or  
 5 by the method of Maxam *et al.*, *Methods in Enzymology* 65:499 (1980).

#### ***Transient expression vectors***

Particularly useful in the practice of this invention are expression vectors that provide for the transient expression in mammalian cells of DNA encoding WSX receptor or OB protein. In general, transient expression involves the use of an expression vector that is able to replicate efficiently in a host cell, such that the host cell  
 10 accumulates many copies of the expression vector and, in turn, synthesizes high levels of a desired polypeptide encoded by the expression vector. Sambrook *et al.*, *supra*, pp. 16.17 - 16.22. Transient expression systems, comprising a suitable expression vector and a host cell, allow for the convenient positive identification of polypeptides encoded by cloned DNAs, as well as for the rapid screening of such polypeptides for desired biological or physiological properties. Thus, transient expression systems are particularly useful in the invention  
 15 for purposes of identifying analogs and variants of WSX receptor or OB protein that are biologically active WSX receptor or OB protein.

#### **(8) *Suitable exemplary vertebrate cell vectors***

Other methods, vectors, and host cells suitable for adaptation to the synthesis of WSX receptor or OB protein in recombinant vertebrate cell culture are described in Gething *et al.*, *Nature* 293:620-625 (1981); Mantel  
 20 *et al.*, *Nature* 281:40-46 (1979); EP 117,060; and EP 117,058. A particularly useful plasmid for mammalian cell culture expression of WSX receptor or OB protein is pRK5 (EP 307,247) or pSVI6B. WO 91/08291 published 13 June 1991.

#### **iii. *Selection and Transformation of Host Cells***

Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast,  
 25 or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, *e.g.*, *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (*e.g.*, *B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One  
 30 preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable. These examples are illustrative rather than limiting. Strain W3110 is a particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell should secrete minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding  
 35 proteins, with examples of such hosts including *E. coli* W3110 strain 27C7. The complete genotype of 27C7 is *tonA*  $\Delta$  *ptr3* *phoA*  $\Delta$  *E15*  $\Delta$ (*argF-lac*)169 *ompT*  $\Delta$  *degP41* *kan<sup>r</sup>*. Strain 27C7 was deposited on 30 October 1991 in the American Type Culture Collection as ATCC No. 55,244. Alternatively, the strain of *E. coli* having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990 may be employed. Alternatively still, methods of cloning, *e.g.*, PCR or other nucleic acid polymerase reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for WSX receptor or OB protein-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe* (Beach *et al.*, *Nature*, 290:140 (1981); EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer *et al.*, *supra*) such as, *e.g.*, *K. lactis* (MW98-8C, CBS683, CBS4574), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilum* (ATCC 36,906; Van den Berg *et al.*, *supra*), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna *et al.*, *J. Basic Microbiol.* 28:265-278 (1988)); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case *et al.*, *Proc. Natl. Acad. Sci. USA* 76:5259-5263 (1979)); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, *e.g.*, *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance *et al.*, *Biochem. Biophys. Res. Commun.* 112:284-289 (1983); Tilburn *et al.*, *Gene* 26:205-221 (1983); Yelton *et al.*, *Proc. Natl. Acad. Sci. USA* 81:1470-1474 (1984)) and *A. niger*. Kelly *et al.*, *EMBO J.* 4:475-479 (1985).

Suitable host cells for the expression of glycosylated WSX receptor or OB protein are derived from multicellular organisms. Such host cells are capable of complex processing and glycosylation activities. In principle, any higher eukaryotic cell culture is workable, whether from vertebrate or invertebrate culture. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. See, *e.g.*, Luckow *et al.*, *Bio/Technology* 6:47-55 (1988); Miller *et al.*, in *Genetic Engineering*, Setlow *et al.*, eds., Vol. 8 (Plenum Publishing, 1986), pp. 277-279; and Maeda *et al.*, *Nature* 315:592-594 (1985). A variety of viral strains for transfection are publicly available, *e.g.*, the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can be utilized as hosts. Typically, plant cells are transfected by incubation with certain strains of the bacterium *Agrobacterium tumefaciens*, which has been previously manipulated to contain the WSX receptor or OB protein-encoding DNA. During incubation of the plant cell culture with *A. tumefaciens*, the DNA encoding the WSX receptor or OB protein is transferred to the plant cell host such that it is transfected, and will, under appropriate conditions, express the WSX receptor or OB protein-encoding DNA. In addition, regulatory and signal sequences compatible with plant cells are available, such as the nopaline synthase promoter and polyadenylation signal sequences. Depicker *et al.*, *J. Mol. Appl. Gen.* 1:561 (1982). In addition, DNA segments isolated from the upstream region of the T-DNA 780 gene are capable of activating or increasing transcription levels of plant-expressible genes in recombinant DNA-containing plant tissue. EP 321,196 published 21 June 1989.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. See, *e.g.*, *Tissue Culture*, Academic Press, Kruse and Patterson, editors (1973). Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40

(COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.* 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (WI38, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather *et al.*, *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors for WSX receptor or OB protein production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example,  $\text{CaPO}_4$  and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in section 1.82 of Sambrook *et al.*, *supra*, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, *Gene* 23:315 (1983) and WO 89/05859 published 29 June 1989. In addition, plants may be transfected using ultrasound treatment as described in WO 91/00358 published 10 January 1991.

For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham *et al.*, *Virology* 52:456-457 (1978) is preferred. General aspects of mammalian cell host system transformations have been described in U.S. Pat. No. 4,399,216 issued 16 August 1983. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, *J. Bact.* 130:946 (1977) and Hsiao *et al.*, *Proc. Natl. Acad. Sci. USA* 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, *etc.*, may also be used. For various techniques for transforming mammalian cells, see Keown *et al.*, *Methods in Enzymology* 185:527-537 (1990) and Mansour *et al.*, *Nature* 336:348-352 (1988).

#### iv. Culturing the Host Cells

Prokaryotic cells used to produce the WSX receptor or OB protein of this invention are cultured in suitable media as described generally in Sambrook *et al.*, *supra*.

The mammalian host cells used to produce the WSX receptor or OB protein of this invention may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma)

are suitable for culturing the host cells. In addition, any of the media described in Ham *et al. Meth. Enz.* 58:44 (1979), Barnes *et al., Anal. Biochem.* 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and thymidine), antibiotics (such as GENTAMYCIN<sup>TM</sup> drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art.

10 The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

In general, principles, protocols, and practical techniques for maximizing the productivity of mammalian cell cultures can be found in *Mammalian Cell Biotechnology: a Practical Approach*, M. Butler, ed. (IRL Press, 1991).

15 The host cells referred to in this disclosure encompass cells in culture as well as cells that are within a host animal.

#### v. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA* 77:5201-5205 (1980)), dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Various labels may be employed, most commonly radioisotopes, particularly <sup>32</sup>P. However, other techniques may also be employed, such as using biotin-modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

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Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. With immunohistochemical staining techniques, a cell sample is prepared, typically by dehydration and fixation, followed by reaction with labeled antibodies specific for the gene product coupled, where the labels are usually visually detectable, such as enzymatic labels, fluorescent labels, luminescent labels, and the like. A particularly sensitive staining technique suitable for use in the present invention is described by Hsu *et al., Am. J. Clin. Path.* 75:734-738 (1980).

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Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared as described herein.

**vi. Purification of WSX Receptor or OB Protein**

WSX receptor (*e.g.*, WSX receptor ECD) or OB protein preferably is recovered from the culture medium as a secreted polypeptide, although it also may be recovered from host cell lysates. If the WSX receptor is membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.* Triton-X 100)

5 When WSX receptor or OB protein is produced in a recombinant cell other than one of human origin, the WSX receptor or OB protein is completely free of proteins or polypeptides of human origin. However, it is necessary to purify WSX receptor or OB protein from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous as to WSX receptor or OB protein. As a first step, the culture medium or lysate is centrifuged to remove particulate cell debris. WSX receptor or OB protein thereafter is  
10 purified from contaminant soluble proteins and polypeptides, with the following procedures being exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75™; and protein A Sepharose™ columns to remove contaminants such as IgG.

15 WSX receptor or OB protein variants in which residues have been deleted, inserted, or substituted are recovered in the same fashion as native sequence WSX receptor or OB protein, taking account of any substantial changes in properties occasioned by the variation. Immunoaffinity columns such as a rabbit polyclonal anti-WSX receptor or OB protein column can be employed to absorb the WSX receptor or OB protein variant by binding it to at least one remaining immune epitope.

20 A protease inhibitor such as phenyl methyl sulfonyl fluoride (PMSF) also may be useful to inhibit proteolytic degradation during purification, and antibiotics may be included to prevent the growth of adventitious contaminants.

**vii. Covalent Modifications**

Covalent modifications of WSX receptor or OB protein are included within the scope of this invention.

25 Both native sequence WSX receptor or OB protein and amino acid sequence variants of the WSX receptor or OB protein may be covalently modified. One type of covalent modification of the WSX receptor or OB protein is introduced into the molecule by reacting targeted amino acid residues of the WSX receptor or OB protein with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues of the WSX receptor or OB protein.

30 Cysteiny l residues most commonly are reacted with  $\alpha$ -haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteiny l residues also are derivatized by reaction with bromotri fluoroacetone,  $\alpha$ -bromo- $\beta$ -(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

35 Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

Lysiny l and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysiny l residues. Other suitable



reagents for derivatizing  $\alpha$ -amino-containing residues include imidoesters such as methyl picolinimide, pyridoxal phosphate, pyridoxal, chloroborohydride, trinitrobenzenesulfonic acid, O-methylisourea, 2,4-pentanedione, and transaminase-catalyzed reaction with glyoxylate.

Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed under alkaline conditions because of the high  $pK_a$  of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as with the arginine epsilon-amino group.

The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidazole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using  $^{125}I$  or  $^{131}I$  to prepare labeled proteins for use in radioimmunoassay, the chloramine T method being suitable.

Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides ( $R-N=C=N-R'$ ), where R and R' are different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

Derivatization with bifunctional agents is useful for crosslinking WSX receptor or OB protein to a water-insoluble support matrix or surface for use in the method for purifying anti-WSX receptor or OB protein antibodies, and vice-versa. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-((p-azidophenyl)dithio)propioimide yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Patent Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. These residues are deamidated under neutral or basic conditions. The deamidated form of these residues falls within the scope of this invention.

Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the  $\alpha$ -amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the WSX receptor or OB protein included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. By altering is meant deleting one or more carbohydrate moieties found in native WSX receptor or OB protein, and/or adding one or more glycosylation sites that are not present in the native WSX receptor or OB protein.

Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxylamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the WSX receptor or OB protein is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the native WSX receptor or OB protein sequence (for O-linked glycosylation sites). For ease, the WSX receptor or OB protein amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the WSX receptor or OB protein at preselected bases such that codons are generated that will translate into the desired amino acids. The DNA mutation(s) may be made using methods described above and in U.S. Pat. No. 5,364,934, *supra*.

Another means of increasing the number of carbohydrate moieties on the WSX receptor or OB protein is by chemical or enzymatic coupling of glycosides to the polypeptide. These procedures are advantageous in that they do not require production of the polypeptide in a host cell that has glycosylation capabilities for N- or O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published 11 September 1987, and in Aplin *et al.*, *CRC Crit. Rev. Biochem.* 259-306 (1981).

Removal of carbohydrate moieties present on the WSX receptor or OB protein may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin, *et al.*, *Arch. Biochem. Biophys.* 259:52 (1987) and by Edge *et al.*, *Anal. Biochem.* 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, *Meth. Enzymol.* 138:350 (1987).

Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin *et al.*, *J. Biol. Chem.* 257:3105 (1982). Tunicamycin blocks the formation of protein-N-glycoside linkages.

Another type of covalent modification of WSX receptor or OB protein comprises linking the WSX receptor or OB protein to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

Since it is often difficult to predict in advance the characteristics of a variant WSX receptor or OB protein, it will be appreciated that some screening of the recovered variant will be needed to select the optimal variant. A change in the immunological character of the WSX receptor or OB protein molecule, such as affinity for a given antibody, is also able to be measured by a competitive-type immunoassay. The WSX receptor variant is assayed for changes in the ability of the protein to induce cell proliferation in the colony assay of Example 8. Other potential modifications of protein or polypeptide properties such as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation, or the tendency to aggregate with carriers or into multimers are assayed by methods well known in the art.

**viii. Epitope-Tagged WSX Receptor or OB Protein**

This invention encompasses chimeric polypeptides comprising WSX receptor or OB protein fused to a heterologous polypeptide. A chimeric WSX receptor or OB protein is one type of WSX receptor or OB protein variant as defined herein. In one preferred embodiment, the chimeric polypeptide comprises a fusion of the WSX receptor or OB protein with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally provided at the amino- or carboxyl- terminus of the WSX receptor or OB protein. Such epitope-tagged forms of the WSX receptor or OB protein are desirable as the presence thereof can be detected using a labeled antibody against the tag polypeptide. Also, provision of the epitope tag enables the WSX receptor or OB protein to be readily purified by affinity purification using the anti-tag antibody. Affinity purification techniques and diagnostic assays involving antibodies are described later herein.

Tag polypeptides and their respective antibodies are well known in the art. Examples include the flu HA tag polypeptide and its antibody 12CA5 (Field *et al.*, *Mol. Cell. Biol.* 8:2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan *et al.*, *Molecular and Cellular Biology* 5:3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody. Paborsky *et al.*, *Protein Engineering* 3(6):547-553 (1990). Other tag polypeptides have been disclosed. Examples include the Flag-peptide (Hopp *et al.*, *BioTechnology* 6:1204-1210 (1988)); the KT3 epitope peptide (Martin *et al.*, *Science* 255:192-194 (1992)); an  $\alpha$ -tubulin epitope peptide (Skinner *et al.*, *J. Biol. Chem.* 266:15163-15166 (1991)); and the T7 gene 10 protein peptide tag. Lutz-Freyermuth *et al.*, *Proc. Natl. Acad. Sci. USA* 87:6393-6397 (1990). Once the tag polypeptide has been selected, an antibody thereto can be generated using the techniques disclosed herein.

The general methods suitable for the construction and production of epitope-tagged WSX receptor or OB protein are the same as those disclosed hereinabove. WSX receptor or OB protein-tag polypeptide fusions are most conveniently constructed by fusing the cDNA sequence encoding the WSX receptor or OB protein portion in-frame to the tag polypeptide DNA sequence and expressing the resultant DNA fusion construct in appropriate host cells. Ordinarily, when preparing the WSX receptor or OB protein-tag polypeptide chimeras of the present invention, nucleic acid encoding the WSX receptor or OB protein will be fused at its 3' end to nucleic acid encoding the N-terminus of the tag polypeptide, however 5' fusions are also possible.

Epitope-tagged WSX receptor or OB protein can be conveniently purified by affinity chromatography using the anti-tag antibody. The matrix to which the affinity antibody is attached is most often agarose, but other matrices are available (e.g. controlled pore glass or poly(styrenedivinyl)benzene). The epitope-tagged WSX

receptor or OB protein can be eluted from the affinity column by varying the buffer pH or ionic strength or adding chaotropic agents, for example.

#### ix. WSX Receptor or OB Protein Immuno adhesins

Chimeras constructed from a receptor sequence linked to an appropriate immunoglobulin constant domain sequence (immuno adhesins) are known in the art. Immuno adhesins reported in the literature include fusions of the T cell receptor\* (Gascoigne *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 2936-2940 (1987)); CD4\* (Capon *et al.*, *Nature* 337: 525-531 (1989); Traunecker *et al.*, *Nature* 339: 68-70 (1989); Zettmeissl *et al.*, *DNA Cell Biol. USA* 9: 347-353 (1990); Byrn *et al.*, *Nature* 344: 667-670 (1990)); L-selectin (homing receptor) ((Watson *et al.*, *J. Cell. Biol.* 110:2221-2229 (1990); Watson *et al.*, *Nature* 349: 164-167 (1991)); CD44\* (Aruffo *et al.*, *Cell* 61: 1303-1313 (1990)); CD28\* and B7\* (Linsley *et al.*, *J. Exp. Med.* 173: 721-730 (1991)); CTLA-4\* (Lisley *et al.*, *J. Exp. Med.* 174: 561-569 (1991)); CD22\* (Stamenkovic *et al.*, *Cell* 66:1133-1144 (1991)); TNF receptor (Ashkenazi *et al.*, *Proc. Natl. Acad. Sci. USA* 88: 10535-10539 (1991); Lesslauer *et al.*, *Eur. J. Immunol.* 27: 2883-2886 (1991); Poppel *et al.*, *J. Exp. Med.* 174:1483-1489 (1991)); NP receptors (Bennett *et al.*, *J. Biol. Chem.* 266:23060-23067 (1991)); and IgE receptor  $\alpha$ \* (Ridgway *et al.*, *J. Cell. Biol.* 115:abstr. 1448 (1991)), where the asterisk (\*) indicates that the receptor is member of the immunoglobulin superfamily.

The simplest and most straightforward immuno adhesin design combines the binding region(s) of the "adhesin" protein with the hinge and Fc regions of an immunoglobulin heavy chain. Ordinarily, when preparing the WSX receptor or OB-immunoglobulin chimeras of the present invention, nucleic acid encoding OB protein or the extracellular domain of the WSX receptor will be fused C-terminally to nucleic acid encoding the N-terminus of an immunoglobulin constant domain sequence, however N-terminal fusions are also possible. For OB-immunoglobulin chimeras, an OB protein fragment which retains the ability to bind to the WSX receptor may be employed.

Typically, in such fusions the encoded chimeric polypeptide will retain at least functionally active hinge, CH2 and CH3 domains of the constant region of an immunoglobulin heavy chain. Fusions are also made to the C-terminus of the Fc portion of a constant domain, or immediately N-terminal to the CH1 of the heavy chain or the corresponding region of the light chain.

The precise site at which the fusion is made is not critical; particular sites are well known and may be selected in order to optimize the biological activity, secretion or binding characteristics of the WSX receptor or OB-immunoglobulin chimeras.

In some embodiments, the WSX receptor or OB-immunoglobulin chimeras are assembled as monomers, or hetero- or homo-multimers, and particularly as dimers or tetramers, essentially as illustrated in WO 91/08298.

In a preferred embodiment, the OB protein sequence or WSX receptor extracellular domain sequence is fused to the N-terminus of the C-terminal portion of an antibody (in particular the Fc domain), containing the effector functions of an immunoglobulin, e.g. immunoglobulin G1 (IgG1). It is possible to fuse the entire heavy chain constant region to the OB protein or WSX receptor extracellular domain sequence. However, more preferably, a sequence beginning in the hinge region just upstream of the papain cleavage site (which defines IgG Fc chemically; residue 216, taking the first residue of heavy chain constant region to be 114, or analogous sites of other immunoglobulins) is used in the fusion. In a particularly preferred embodiment, the OB protein or WSX

receptor amino acid sequence is fused to the hinge region, CH2 and CH3, or the CH1, hinge, CH2 and CH3 domains of an IgG1, IgG2, or IgG3 heavy chain. The precise site at which the fusion is made is not critical, and the optimal site can be determined by routine experimentation.

In some embodiments, the WSX receptor or OB-immunoglobulin chimeras are assembled as multimers, and particularly as homo-dimers or -tetramers. Generally, these assembled immunoglobulins will have known unit structures. A basic four chain structural unit is the form in which IgG, IgD, and IgE exist. A four unit is repeated in the higher molecular weight immunoglobulins; IgM generally exists as a pentamer of basic four units held together by disulfide bonds. IgA globulin, and occasionally IgG globulin, may also exist in multimeric form in serum. In the case of multimer, each four unit may be the same or different.

Various exemplary assembled WSX receptor or OB-immunoglobulin chimeras within the scope herein are schematically diagrammed below:

- (a)  $AC_L-AC_L$ ;
- (b)  $AC_H-(AC_H, AC_L-AC_H, AC_L-V_HCH, \text{ or } V_LC_L-AC_H)$ ;
- (c)  $AC_L-AC_H-(AC_L-AC_H, AC_L-V_HCH, V_LC_L-AC_H, \text{ or } V_LC_L-V_HCH)$ ;
- (d)  $AC_L-V_HCH-(AC_H, \text{ or } AC_L-V_HCH, \text{ or } V_LC_L-AC_H)$ ;
- (e)  $V_LC_L-AC_H-(AC_L-V_HCH, \text{ or } V_LC_L-AC_H)$ ; and
- (f)  $(A-Y)_n-(V_LC_L-V_HCH)_2$ ,

wherein

- each A represents identical or different OB protein or WSX receptor amino acid sequences;
- $V_L$  is an immunoglobulin light chain variable domain;
- $V_H$  is an immunoglobulin heavy chain variable domain;
- $C_L$  is an immunoglobulin light chain constant domain;
- $C_H$  is an immunoglobulin heavy chain constant domain;
- n is an integer greater than 1;
- Y designates the residue of a covalent cross-linking agent.

In the interests of brevity, the foregoing structures only show key features; they do not indicate joining (J) or other domains of the immunoglobulins, nor are disulfide bonds shown. However, where such domains are required for binding activity, they shall be constructed as being present in the ordinary locations which they occupy in the immunoglobulin molecules.

Alternatively, the OB protein or WSX receptor extracellular domain sequence can be inserted between immunoglobulin heavy chain and light chain sequences such that an immunoglobulin comprising a chimeric heavy chain is obtained. In this embodiment, the OB protein or WSX receptor sequence is fused to the 3' end of an immunoglobulin heavy chain in each arm of an immunoglobulin, either between the hinge and the CH2 domain, or between the CH2 and CH3 domains. Similar constructs have been reported by Hoogenboom *et al.*, *Mol. Immunol.*, 28:1027-1037 (1991).

Although the presence of an immunoglobulin light chain is not required in the immunoadhesins of the present invention, an immunoglobulin light chain might be present either covalently associated to an OB protein or WSX receptor-immunoglobulin heavy chain fusion polypeptide, or directly fused to the WSX receptor extracellular domain or OB protein. In the former case, DNA encoding an immunoglobulin light chain is

typically coexpressed with the DNA encoding the OB protein or WSX receptor-immunoglobulin heavy chain fusion protein. Upon secretion, the hybrid heavy chain and the light chain will be covalently associated to provide an immunoglobulin-like structure comprising two disulfide-linked immunoglobulin heavy chain-light chain pairs. Methods suitable for the preparation of such structures are, for example, disclosed in U.S. Patent  
5 No. 4,816,567 issued 28 March 1989.

In a preferred embodiment, the immunoglobulin sequences used in the construction of the immunoadhesins of the present invention are from an IgG immunoglobulin heavy chain constant domain. For human immunoadhesins, the use of human IgG1 and IgG3 immunoglobulin sequences is preferred. A major advantage of using IgG1 is that IgG1 immunoadhesins can be purified efficiently on immobilized protein A. In  
10 contrast, purification of IgG3 requires protein G, a significantly less versatile medium. However, other structural and functional properties of immunoglobulins should be considered when choosing the Ig fusion partner for a particular immunoadhesin construction. For example, the IgG3 hinge is longer and more flexible, so it can accommodate larger adhesion domains that may not fold or function properly when fused to IgG1. Another consideration may be valency; IgG immunoadhesins are bivalent homodimers, whereas Ig subtypes like IgA and  
15 IgM may give rise to dimeric or pentameric structures, respectively, of the basic Ig homodimer unit. For immunoadhesins designed for *in vivo* application, the pharmacokinetic properties and the effector functions specified by the Fc region are important as well. Although IgG1, IgG2 and IgG4 all have *in vivo* half-lives of 21 days, their relative potencies at activating the complement system are different. IgG4 does not activate complement, and IgG2 is significantly weaker at complement activation than IgG1. Moreover, unlike IgG1,  
20 IgG2 does not bind to Fc receptors on mononuclear cells or neutrophils. While IgG3 is optimal for complement activation, its *in vivo* half-life is approximately one third of the other IgG isotypes. Another important consideration for immunoadhesins designed to be used as human therapeutics is the number of allotypic variants of the particular isotype. In general, IgG isotypes with fewer serologically-defined allotypes are preferred. For example, IgG1 has only four serologically-defined allotypic sites, two of which (G1m and 2) are located in the  
25 Fc region; and one of these sites G1m1, is non-immunogenic. In contrast, there are 12 serologically-defined allotypes in IgG3, all of which are in the Fc region; only three of these sites (G3m5, 11 and 21) have one allotype which is nonimmunogenic. Thus, the potential immunogenicity of a  $\gamma 3$  immunoadhesin is greater than that of a  $\gamma 1$  immunoadhesin.

With respect to the parental immunoglobulin, a useful joining point is just upstream of the cysteines of  
30 the hinge that form the disulfide bonds between the two heavy chains. In a frequently used design, the codon for the C-terminal residue of the WSX receptor or OB protein part of the molecule is placed directly upstream of the codons for the sequence DKTHTCPPCP (SEQ ID NO:44) of the IgG1 hinge region.

The general methods suitable for the construction and expression of immunoadhesins are the same as those disclosed hereinabove with regard to WSX receptor and OB protein. Immunoadhesins are most  
35 conveniently constructed by fusing the cDNA sequence encoding the WSX receptor or OB protein portion in-frame to an Ig cDNA sequence. However, fusion to genomic Ig fragments can also be used (see, e.g., Gascoigne *et al.*, *Proc. Natl. Acad. Sci. USA*, 84:2936-2940 (1987); Aruffo *et al.*, *Cell* 61:1303-1313 (1990); Stamenkovic *et al.*, *Cell* 66:1133-1144 (1991)). The latter type of fusion requires the presence of Ig regulatory sequences for expression. cDNAs encoding IgG heavy-chain constant regions can be isolated based on published sequence

from cDNA libraries derived from spleen or peripheral blood lymphocytes, by hybridization or by polymerase chain reaction (PCR) techniques. The cDNAs encoding the WSX receptor or OB protein and Ig parts of the immunoadhesin are inserted in tandem into a plasmid vector that directs efficient expression in the chosen host cells. For expression in mammalian cells, pRK5-based vectors (Schall *et al.*, *Cell* 61:361-370 (1990)) and  
5 CDM8-based vectors (Seed, *Nature* 329:840 (1989)) can be used. The exact junction can be created by removing the extra sequences between the designed junction codons using oligonucleotide-directed deletional mutagenesis (Zoller *et al.*, *Nucleic Acids Res.* 10:6487 (1982); Capon *et al.*, *Nature* 337:525-531 (1989)). Synthetic oligonucleotides can be used, in which each half is complementary to the sequence on either side of the desired junction; ideally, these are 36 to 48-mers. Alternatively, PCR techniques can be used to join the two  
10 parts of the molecule in-frame with an appropriate vector.

The choice of host cell line for the expression of the immunoadhesin depends mainly on the expression vector. Another consideration is the amount of protein that is required. Milligram quantities often can be produced by transient transfections. For example, the adenovirus EIA-transformed 293 human embryonic kidney cell line can be transfected transiently with pRK5-based vectors by a modification of the calcium phosphate  
15 method to allow efficient immunoadhesin expression. CDM8-based vectors can be used to transfect COS cells by the DEAE-dextran method (Aruffo *et al.*, *Cell* 61:1303-1313 (1990); Zettmeissl *et al.*, *DNA Cell Biol. US* 9:347-353 (1990)). If larger amounts of protein are desired, the immunoadhesin can be expressed after stable transfection of a host cell line. For example, a pRK5-based vector can be introduced into Chinese hamster ovary (CHO) cells in the presence of an additional plasmid encoding dihydrofolate reductase (DHFR) and conferring  
20 resistance to G418. Clones resistant to G418 can be selected in culture; these clones are grown in the presence of increasing levels of DHFR inhibitor methotrexate; clones are selected, in which the number of gene copies encoding the DHFR and immunoadhesin sequences is co-amplified. If the immunoadhesin contains a hydrophobic leader sequence at its N-terminus, it is likely to be processed and secreted by the transfected cells. The expression of immunoadhesins with more complex structures may require uniquely suited host cells; for  
25 example, components such as light chain or J chain may be provided by certain myeloma or hybridoma cell hosts (Gascoigne *et al.*, 1987, *supra*, Martin *et al.*, *J. Virol.* 67:3561-3568 (1993)).

Immunoadhesins can be conveniently purified by affinity chromatography. The suitability of protein A as an affinity ligand depends on the species and isotype of the immunoglobulin Fc domain that is used in the chimera. Protein A can be used to purify immunoadhesins that are based on human  $\gamma 1$ ,  $\gamma 2$ , or  $\gamma 4$  heavy chains  
30 (Lindmark *et al.*, *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human  $\gamma 3$  (Guss *et al.*, *EMBO J.* 5:1567-1575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. The conditions for binding an immunoadhesin to the protein A or G affinity column are dictated  
35 entirely by the characteristics of the Fc domain; that is, its species and isotype. Generally, when the proper ligand is chosen, efficient binding occurs directly from unconditioned culture fluid. One distinguishing feature of immunoadhesins is that, for human  $\gamma 1$  molecules, the binding capacity for protein A is somewhat diminished relative to an antibody of the same Fc type. Bound immunoadhesin can be efficiently eluted either at acidic pH

(at or above 3.0), or in a neutral pH buffer containing a mildly chaotropic salt. This affinity chromatography step can result in an immunoadhesin preparation that is >95% pure.

Other methods known in the art can be used in place of, or in addition to, affinity chromatography on protein A or G to purify immunoadhesins. Immunoadhesins behave similarly to antibodies in thiophilic gel chromatography (Hutchens *et al.*, *Anal. Biochem.* 159:217-226 (1986)) and immobilized metal chelate chromatography (Al-Mashikhi *et al.*, *J. Dairy Sci.* 71:1756-1763 (1988)). In contrast to antibodies, however, their behavior on ion exchange columns is dictated not only by their isoelectric points, but also by a charge dipole that may exist in the molecules due to their chimeric nature.

If desired, the immunoadhesins can be made bispecific. Thus, the immunoadhesins of the present invention may combine a WSX receptor extracellular domain and a domain, such as the extracellular domain, of another cytokine receptor subunit. Exemplary cytokine receptors from which such bispecific immunoadhesin molecules can be made include TPO (or *mpl* ligand), EPO, G-CSF, IL-4, IL-7, GH, PRL, IL-3, GM-CSF, IL-5, IL-6, LIF, OSM, CNTF and IL-2 receptors. Alternatively, an OB protein domain may be combined with another cytokine, such as those exemplified herein, in the generation of a bispecific immunoadhesin. For bispecific molecules, trimeric molecules, composed of a chimeric antibody heavy chain in one arm and a chimeric antibody heavy chain-light chain pair in the other arm of their antibody-like structure are advantageous, due to ease of purification. In contrast to antibody-producing quadromas traditionally used for the production of bispecific immunoadhesins, which produce a mixture of ten tetramers, cells transfected with nucleic acid encoding the three chains of a trimeric immunoadhesin structure produce a mixture of only three molecules, and purification of the desired product from this mixture is correspondingly easier.

#### x. Long Half-Life Derivatives of OB Protein

Preferred OB protein functional derivatives for use in the methods of the present invention include OB-immunoglobulin chimeras (immunoadhesins) and other longer half-life molecules. Techniques for generating OB protein immunoadhesins have been described above. The preferred OB immunoadhesin is made according to the techniques described in Example 11 below.

Other derivatives of the OB proteins, which possess a longer half-life than the native molecules comprise the OB protein or an OB-immunoglobulin chimera covalently bonded to a nonproteinaceous polymer. The nonproteinaceous polymer ordinarily is a hydrophilic synthetic polymer, *i.e.*, a polymer not otherwise found in nature. However, polymers which exist in nature and are produced by recombinant or *in vitro* methods are useful, as are polymers which are isolated from native sources. Hydrophilic polyvinyl polymers fall within the scope of this invention, *e.g.* polyvinylalcohol and polyvinylpyrrolidone. Particularly useful are polyalkylene ethers such as polyethylene glycol (PEG); polyelkylenes such as polyoxyethylene, polyoxypropylene, and block copolymers of polyoxyethylene and polyoxypropylene (Pluronic<sup>TM</sup>); polymethacrylates; carbomers; branched or unbranched polysaccharides which comprise the saccharide monomers D-mannose, D- and L-galactose, fucose, fructose, D-xylose, L-arabinose, D-glucuronic acid, sialic acid, D-galacturonic acid, D-mannuronic acid (*e.g.* polymannuronic acid, or alginic acid), D-glucosamine, D-galactosamine, D-glucose and neuraminic acid including homopolysaccharides and heteropolysaccharides such as lactose, amylopectin, starch, hydroxyethyl starch, amylose, dextrane sulfate, dextran, dextrans, glycogen, or the polysaccharide subunit of acid mucopolysaccharides, *e.g.* hyaluronic acid; polymers of sugar alcohols such as polysorbitol and polymannitol;



heparin or heparon. The polymer prior to cross-linking need not be, but preferably is, water soluble, but the final conjugate must be water soluble. In addition, the polymer should not be highly immunogenic in the conjugate form, nor should it possess viscosity that is incompatible with intravenous infusion or injection if it is intended to be administered by such routes.

5            Preferably the polymer contains only a single group which is reactive. This helps to avoid cross-linking of protein molecules. However, it is within the scope herein to optimize reaction conditions to reduce cross-linking, or to purify the reaction products through gel filtration or chromatographic sieves to recover substantially homogenous derivatives.

10           The molecular weight of the polymer may desirably range from about 100 to 500,000, and preferably is from about 1,000 to 20,000. The molecular weight chosen will depend upon the nature of the polymer and the degree of substitution. In general, the greater the hydrophilicity of the polymer and the greater the degree of substitution, the lower the molecular weight that can be employed. Optimal molecular weights will be determined by routine experimentation.

15           The polymer generally is covalently linked to the OB protein or to the OB-immunoglobulin chimera though a multifunctional crosslinking agent which reacts with the polymer and one or more amino acid or sugar residues of the OB protein or OB-immunoglobulin chimera to be linked. However, it is within the scope of the invention to directly crosslink the polymer by reacting a derivatized polymer with the hybrid, or via versa.

20           The covalent crosslinking site on the OB protein or OB-immunoglobulin chimera includes the N-terminal amino group and epsilon amino groups found on lysine residues, as well as other amino, imino, carboxyl, sulfhydryl, hydroxyl or other hydrophilic groups. The polymer may be covalently bonded directly to the hybrid without the use of a multifunctional (ordinarily bifunctional) crosslinking agent. Covalent binding to amino groups is accomplished by known chemistries based upon cyanuric chloride, carbonyl diimidazole, aldehyde reactive groups (PEG alkoxide plus diethyl acetal of bromoacetaldehyde; PEG plus DMSO and acetic anhydride, or PEG chloride plus the phenoxide of 4-hydroxybenzaldehyde, succinimidyl active esters, activated dithiocarbonate PEG, 2,4,5-trichlorophenylchloroformate or P-nitrophenylchloroformate activated PEG). Carboxyl groups are derivatized by coupling PEG-amine using carbodiimide.

25           Polymers are conjugated to oligosaccharide groups by oxidation using chemicals, *e.g.* metaperiodate, or enzymes, *e.g.* glucose or galactose oxidase (either of which produces the aldehyde derivative of the carbohydrate), followed by reaction with hydrazide or amino derivatized polymers, in the same fashion as is described by Heitzmann *et al.*, *P.N.A.S.* 71:3537-41 (1974) or Bayer *et al.*, *Methods in Enzymology* 62:310 (1979), for the labeling of oligosaccharides with biotin or avidin. Further, other chemical or enzymatic methods which have been used heretofore to link oligosaccharides are particularly advantageous because, in general, there are fewer substitutions than amino acid sites for derivatization, and the oligosaccharide products thus will be more homogenous. The oligosaccharide substituents also are optionally modified by enzyme digestion to remove sugars, *e.g.* by neuraminidase digestion, prior to polymer derivatization.

35           The polymer will bear a group which is directly reactive with an amino acid side chain, or the N- or C-terminus of the polypeptide linked, or which is reactive with the multifunctional cross-linking agent. In general, polymers bearing such reactive groups are known for the preparation of immobilized proteins. In order to use such chemistries here, one should employ a water soluble polymer otherwise derivatized in the same fashion as

insoluble polymers heretofore employed for protein immobilization. Cyanogen bromide activation is a particularly useful procedure to employ in crosslinking polysaccharides.

"Water soluble" in reference to the starting polymer means that the polymer or its reactive intermediate used for conjugation is sufficiently water soluble to participate in a derivatization reaction.

- 5 "Water soluble" in reference to the polymer conjugate means that the conjugate is soluble in physiological fluids such as blood.

The degree of substitution with such a polymer will vary depending upon the number of reactive sites on the protein, whether all or a fragment of the protein is used, whether the protein is a fusion with a heterologous protein (e.g. an OB-immunoglobulin chimera), the molecular weight, hydrophilicity and other characteristics  
10 of the polymer, and the particular protein derivatization sites chosen. In general, the conjugate contains about from 1 to 10 polymer molecules, while any heterologous sequence may be substituted with an essentially unlimited number of polymer molecules so long as the desired activity is not significantly adversely affected. The optimal degree of cross-linking is easily determined by an experimental matrix in which the time, temperature and other reaction conditions are varied to change the degree of substitution, after which the ability  
15 of the conjugates to function in the desired fashion is determined.

The polymer, e.g. PEG, is cross-linked by a wide variety of methods known *per se* for the covalent modification of proteins with nonproteinaceous polymers such as PEG. Certain of these methods, however, are not preferred for the purposes herein. Cyanuronic chloride chemistry leads to many side reactions, including protein cross-linking. In addition, it may be particularly likely to lead to inactivation of proteins containing  
20 sulfhydryl groups. Carbonyl diimidazole chemistry (Beauchamp *et al.*, *Anal Biochem.* 131:25-33 (1983)) requires high pH (>8.5), which can inactivate proteins. Moreover, since the "activated PEG" intermediate can react with water, a very large molar excess of "activated PEG" over protein is required. The high concentrations of PEG required for the carbonyl diimidazole chemistry also led to problems in purification, as both gel filtration chromatography and hydrophilic interaction chromatography are adversely affected. In addition, the high  
25 concentrations of "activated PEG" may precipitate protein, a problem that *per se* has been noted previously (Davis, U.S. Patent No. 4,179,337). On the other hand, aldehyde chemistry (Royer, U.S. Patent No. 4,002,531) is more efficient since it requires only a 40-fold molar excess of PEG and a 1-2 hr incubation. However, the manganese dioxide suggested by Royer for preparation of the PEG aldehyde is problematic "because of the pronounced tendency of PEG to form complexes with metal-based oxidizing agents" (Harris *et al.*, *J. Polym.*  
30 *Sci. Polym. Chem. Ed.* 22:341-52 (1984)). The use of a Moffatt oxidation, utilizing DMSO and acetic anhydride, obviates this problem. In addition, the sodium borohydride suggested by Royer must be used at high pH and has a significant tendency to reduce disulfide bonds. In contrast, sodium cyanoborohydride, which is effective at neutral pH and has very little tendency to reduce disulfide bonds is preferred.

Functionalized PEG polymers to modify the OB protein or OB-immunoglobulin chimeras of the present  
35 invention are available from Shearwater Polymers, Inc. (Huntsville, AL). Such commercially available PEG derivatives include, but are not limited to, amino-PEG, PEG amino acid esters, PEG-hydrazide, PEG-thiol, PEG-succinate, carboxymethylated PEG, PEG-propionic acid, PEG amino acids, PEG succinimidyl succinate, PEG succinimidyl propionate, succinimidyl ester of carboxymethylated PEG, succinimidyl carbonate of PEG, succinimidyl esters of amino acid PEGs, PEG-oxycarbonylimidazole, PEG-nitrophenyl carbonate, PEG tresylate,

PEG-glycidyl ether, PEG-aldehyde, PEG vinylsulfone, PEG-maleimide, PEG-orthopyridyl-disulfide, heterofunctional PEGs, PEG vinyl derivatives, PEG silanes, and PEG phospholides. The reaction conditions for coupling these PEG derivatives will vary depending on the protein, the desired degree of PEGylation, and the PEG derivative utilized. Some factors involved in the choice of PEG derivatives include: the desired point of attachment (lysine or cysteine), hydrolytic stability and reactivity of the derivatives, stability, toxicity and antigenicity of the linkage, suitability for analysis, etc. Specific instructions for the use of any particular derivative are available from the manufacturer.

The long half-life conjugates of this invention are separated from the unreacted starting materials by gel filtration. Heterologous species of the conjugates are purified from one another in the same fashion. The polymer also may be water-insoluble, as a hydrophilic gel.

The conjugates may also be purified by ion-exchange chromatography. The chemistry of many of the electrophilically activated PEG's results in a reduction of amino group charge of the PEGylated product. Thus, high resolution ion exchange chromatography can be used to separate the free and conjugated proteins, and to resolve species with different levels of PEGylation. In fact, the resolution of different species (*e.g.* containing one or two PEG residues) is also possible due to the difference in the ionic properties of the unreacted amino acids.

#### **B. Therapeutic Uses for the WSX Receptor**

The WSX receptor and WSX receptor gene are believed to find therapeutic use for administration to a mammal in the treatment of diseases characterized by a decrease in hematopoietic cells. Examples of these diseases include: anemia (including macrocytic and aplastic anemia); thrombocytopenia; hypoplasia; disseminated intravascular coagulation (DIC); myelodysplasia; immune (autoimmune) thrombocytopenic purpura (ITP); and HIV induced ITP. Additionally, these WSX receptor molecules may be useful in treating myeloproliferative thrombocytotic diseases as well as thrombocytosis from inflammatory conditions and in iron deficiency. WSX receptor polypeptide and WSX receptor gene which lead to an increase in hematopoietic cell proliferation may also be used to enhance repopulation of mature blood cell lineages in cells having undergone chemo- or radiation therapy or bone marrow transplantation therapy. Generally, the WSX receptor molecules are expected to lead to an enhancement of the proliferation and/or differentiation (but especially proliferation) of primitive hematopoietic cells. Other potential therapeutic applications for WSX receptor and WSX receptor gene include the treatment of obesity and diabetes and for promoting kidney, liver and lung growth and/or repair (*e.g.* in renal failure). WSX receptor can also be used to treat obesity-related conditions, such as type II adult onset diabetes, infertility, hypercholesterolemia, hyperlipidemia, cardiovascular disease and hypertension.

The WSX receptor may be administered alone or in combination with cytokines (such as OB protein), growth factors or antibodies in the above-identified clinical situations. This may facilitate an effective lowering of the dose of WSX receptor. Suitable dosages for such additional molecules will be discussed below.

Administration of WSX receptor to a mammal having depressed levels of endogenous WSX receptor or a defective WSX receptor gene is contemplated, preferably in the situation where such depressed levels lead to a pathological disorder, or where there is lack of activation of the WSX receptor. In these embodiments where the full length WSX receptor is to be administered to the patient, it is contemplated that the gene encoding the receptor may be administered to the patient via gene therapy technology.

In gene therapy applications, genes are introduced into cells in order to achieve *in vivo* synthesis of a therapeutically effective genetic product, for example for replacement of a defective gene. "Gene therapy" includes both conventional gene therapy where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective DNA or mRNA. Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes *in vivo*. It has already been shown that short antisense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane. (Zamecnik *et al.*, *Proc. Natl. Acad. Sci. USA*, 83:4143-4146 (1986)). The oligonucleotides can be modified to enhance their uptake, *e.g.*, by substituting their negatively charged phosphodiester groups by uncharged groups.

There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, etc. The currently preferred *in vivo* gene transfer techniques include transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau *et al.*, *Trends in Biotechnology* 11:205-210 (1993)). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, etc. Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.* capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, and proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, *J. Biol. Chem.* 262:4429-4432 (1987); and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 87:3410-3414 (1990). For review of the currently known gene marking and gene therapy protocols see Anderson *et al.*, *Science* 256:808-813 (1992).

The invention also provides antagonists of WSX receptor activation (*e.g.* WSX receptor ECD, WSX receptor immunoadhesins and WSX receptor antisense nucleic acid; neutralizing antibodies and uses thereof are discussed in section E below). Administration of WSX receptor antagonist to a mammal having increased or excessive levels of endogenous WSX receptor activation is contemplated, preferably in the situation where such levels of WSX receptor activation lead to a pathological disorder.

In one embodiment, WSX receptor antagonist molecules may be used to bind endogenous ligand in the body, thereby causing desensitized WSX receptors to become responsive to WSX ligand, especially when the levels of WSX ligand in the serum exceed normal physiological levels. Also, it may be beneficial to bind endogenous WSX ligand which is activating undesired cellular responses (such as proliferation of tumor cells). Potential therapeutic applications for WSX antagonists include for example, treatment of metabolic disorders (*e.g.*, anorexia, cachexia, steroid-induced truncal obesity and other wasting diseases characterized by loss of appetite, diminished food intake or body weight loss), stem cell tumors and other tumors which express WSX receptor.

Pharmaceutical compositions of the WSX receptor ECD may further include a WSX ligand. Such dual compositions may be beneficial where it is therapeutically useful to prolong half-life of WSX ligand, and/or activate endogenous WSX receptor directly as a heterotrimeric complex.

Therapeutic formulations of WSX receptor are prepared for storage by mixing WSX receptor having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (*Remington's Pharmaceutical Sciences*, 16th edition, Osol, A., Ed., (1980)), in the form of lyophilized cake or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counter-ions such as sodium; and/or non-ionic surfactants such as Tween, Plurionics™ or polyethylene glycol (PEG).

The WSX receptor also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules), or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences*, *supra*.

WSX receptor to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. WSX receptor ordinarily will be stored in lyophilized form or in solution.

Therapeutic WSX receptor compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of WSX receptor administration is in accord with known methods, *e.g.*, those routes set forth above for specific indications, as well as the general routes of injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional means, or sustained release systems as noted below. WSX receptor is administered continuously by infusion or by bolus injection. Generally, where the disorder permits, one should formulate and dose the WSX receptor for site-specific delivery.

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the protein, which matrices are in the form of shaped articles, *e.g.*, films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (*e.g.*, poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*, *J. Biomed. Mater. Res.* 15:167-277 (1981) and Langer, *Chem. Tech.* 12:98-105 (1982) or poly(vinylalcohol)), polylactides (U.S. Patent No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and  $\gamma$  ethyl-L-glutamate (Sidman *et al.*, *Biopolymers* 22:547-556 (1983)), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the Lupron

Depot<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988).

While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated proteins remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for protein stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release WSX receptor compositions also include liposomally entrapped WSX receptor. Liposomes containing WSX receptor are prepared by methods known *per se*: DE 3,218,121; Epstein *et al.*, *Proc. Natl. Acad. Sci. USA* 82:3688-3692 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. % cholesterol, the selected proportion being adjusted for the optimal WSX receptor therapy.

When applied topically, the WSX receptor is suitably combined with other ingredients, such as carriers and/or adjuvants. There are no limitations on the nature of such other ingredients, except that they must be physiologically acceptable and efficacious for their intended administration, and cannot degrade the activity of the active ingredients of the composition. Examples of suitable vehicles include ointments, creams, gels, or suspensions, with or without purified collagen. The compositions also may be impregnated into transdermal patches, plasters, and bandages, preferably in liquid or semi-liquid form.

For obtaining a gel formulation, the WSX receptor formulated in a liquid composition may be mixed with an effective amount of a water-soluble polysaccharide or synthetic polymer such as PEG to form a gel of the proper viscosity to be applied topically. The polysaccharide that may be used includes, for example, cellulose derivatives such as etherified cellulose derivatives, including alkyl celluloses, hydroxyalkyl celluloses, and alkylhydroxyalkyl celluloses, for example, methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl methylcellulose, and hydroxypropyl cellulose; starch and fractionated starch; agar; alginic acid and alginates; gum arabic; pullullan; agarose; carrageenan; dextrans; dextrans; fructans; inulin; mannans; xylans; arabinans; chitosans; glycogens; glucans; and synthetic biopolymers; as well as gums such as xanthan gum; guar gum; locust bean gum; gum arabic; tragacanth gum; and karaya gum; and derivatives and mixtures thereof. The preferred gelling agent herein is one that is inert to biological systems, nontoxic, simple to prepare, and not too runny or viscous, and will not destabilize the WSX receptor held within it.

Preferably the polysaccharide is an etherified cellulose derivative, more preferably one that is well defined, purified, and listed in USP, *e.g.*, methylcellulose and the hydroxyalkyl cellulose derivatives, such as hydroxypropyl cellulose, hydroxyethyl cellulose, and hydroxypropyl methylcellulose. Most preferred herein is methylcellulose.

The polyethylene glycol useful for gelling is typically a mixture of low and high molecular weight PEGs to obtain the proper viscosity. For example, a mixture of a PEG of molecular weight 400-600 with one of molecular weight 1500 would be effective for this purpose when mixed in the proper ratio to obtain a paste.

The term "water soluble" as applied to the polysaccharides and PEGs is meant to include colloidal solutions and dispersions. In general, the solubility of the cellulose derivatives is determined by the degree of substitution of ether groups, and the stabilizing derivatives useful herein should have a sufficient quantity of such ether groups per anhydroglucose unit in the cellulose chain to render the derivatives water soluble. A degree of ether substitution of at least 0.35 ether groups per anhydroglucose unit is generally sufficient. Additionally, the cellulose derivatives may be in the form of alkali metal salts, for example, the Li, Na, K, or Cs salts.

If methylcellulose is employed in the gel, preferably it comprises about 2-5%, more preferably about 3%, of the gel and the WSX receptor is present in an amount of about 300-1000 mg per ml of gel.

An effective amount of WSX receptor to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the WSX receptor until a dosage is reached that achieves the desired effect. A typical daily dosage for systemic treatment might range from about 1 µg/kg to up to 10 mg/kg or more, depending on the factors mentioned above. As an alternative general proposition, the WSX receptor is formulated and delivered to the target site or tissue at a dosage capable of establishing in the tissue a WSX receptor level greater than about 0.1 ng/cc up to a maximum dose that is efficacious but not unduly toxic. This intra-tissue concentration should be maintained if possible by continuous infusion, sustained release, topical application, or injection at empirically determined frequencies. The progress of this therapy is easily monitored by conventional assays.

### C. Non-Therapeutic Uses for the WSX Receptor

WSX receptor nucleic acid is useful for the preparation of WSX receptor polypeptide by recombinant techniques exemplified herein which can then be used for production of anti-WSX receptor antibodies having various utilities described below.

The WSX receptor (polypeptide or nucleic acid) can be used to induce proliferation and/or differentiation of cells *in vitro*. In particular, it is contemplated that this molecule may be used to induce proliferation of stem cell/progenitor cell populations (e.g. CD34+ cell populations obtained as described in Example 8 below). These cells which are to be grown *ex vivo* may simultaneously be exposed to other known growth factors or cytokines, such as those described herein. This results in proliferation and/or differentiation of the cells having the WSX receptor.

In yet another aspect of the invention, the WSX receptor may be used for affinity purification of WSX ligand. Briefly, this technique involves: (a) contacting a source of WSX ligand with an immobilized WSX receptor under conditions whereby the WSX ligand to be purified is selectively adsorbed onto the immobilized receptor; (b) washing the immobilized WSX receptor and its support to remove non-adsorbed material; and (c) eluting the WSX ligand molecules from the immobilized WSX receptor to which they are adsorbed with an elution buffer. In a particularly preferred embodiment of affinity purification, WSX receptor is covalently attaching to an inert and porous matrix (e.g., agarose reacted with cyanogen bromide). Especially preferred is

a WSX receptor immunoadhesin immobilized on a protein A column. A solution containing WSX ligand is then passed through the chromatographic material. The WSX ligand adsorbs to the column and is subsequently released by changing the elution conditions (*e.g.* by changing pH or ionic strength).

The WSX receptor may be used for competitive screening of potential agonists or antagonists for binding to the WSX receptor. Such agonists or antagonists may constitute potential therapeutics for treating conditions characterized by insufficient or excessive WSX receptor activation, respectively.

The preferred technique for identifying molecules which bind to the WSX receptor utilizes a chimeric receptor (*e.g.*, epitope tagged WSX receptor or WSX receptor immunoadhesin) attached to a solid phase, such as the well of an assay plate. Binding of molecules which are optionally labelled (*e.g.*, radiolabelled) to the immobilized receptor can be evaluated.

To identify WSX receptor agonists or antagonists, the thymidine incorporation assay can be used. For screening for antagonists, the WSX receptor can be exposed to a WSX ligand followed by the putative antagonist, or the WSX ligand and antagonist can be added to the WSX receptor simultaneously, and the ability of the antagonist to block receptor activation can be evaluated.

The WSX receptor polypeptides are also useful as molecular weight markers. To use a WSX receptor polypeptide as a molecular weight marker, gel filtration chromatography or SDS-PAGE, for example, will be used to separate protein(s) for which it is desired to determine their molecular weight(s) in substantially the normal way. The WSX receptor and other molecular weight markers will be used as standards to provide a range of molecular weights. For example, phosphorylase b (mw = 97,400), bovine serum albumin (mw = 68,000), ovalbumin (mw = 46,000), WSX receptor (mw = 44,800), trypsin inhibitor (mw = 20,100), and lysozyme (mw = 14,400) can be used as mw markers. The other molecular weight markers mentioned here can be purchased commercially from Amersham Corporation, Arlington Heights, IL. The molecular weight markers are generally labeled to facilitate detection thereof. For example, the markers may be biotinylated and following separation can be incubated with streptavidin-horseradish peroxidase so that the various markers can be detected by light detection.

The purified WSX receptor, and the nucleic acid encoding it, may also be sold as reagents for mechanism studies of WSX receptor and its ligands, to study the role of the WSX receptor and WSX ligand in normal growth and development, as well as abnormal growth and development, *e.g.*, in malignancies.

WSX receptor variants are useful as standards or controls in assays for the WSX receptor for example ELISA, RIA, or RRA, provided that they are recognized by the analytical system employed, *e.g.*, an anti-WSX receptor antibody.

#### **D. WSX Receptor Antibody Preparation**

##### **1. Polyclonal antibodies**

Polyclonal antibodies are generally raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. In that the preferred epitope is in the ECD of the WSX receptor, it is desirable to use WSX receptor ECD or a molecule comprising the ECD (*e.g.*, WSX receptor immunoadhesin) as the antigen for generation of polyclonal and monoclonal antibodies. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or



derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride,  $\text{SOCl}_2$ , or  $\text{R}^1\text{N}=\text{C}=\text{NR}$ , where R and  $\text{R}^1$  are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining 1 mg or 1  $\mu\text{g}$  of the peptide or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

## 2. Monoclonal antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler *et al.*, *Nature* 256:495 (1975), or may be made by recombinant DNA methods (Cabilly *et al.*, *supra*).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 cells available from the American Type Culture Collection, Rockville, Maryland USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.* 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

5       The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson *et al.*, *Anal. Biochem.* 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *supra*). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In  
10       addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional  
15       procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells.  
20       Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra *et al.*, *Curr. Opinion in Immunol.* 5:256-262 (1993) and Plückthun, *Immunol. Revs.* 130:151-188 (1992).

In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty *et al.*, *Nature* 348:552-554 (1990). Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991) describe the isolation of murine  
25       and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Mark *et al.*, *Bio/Technology* 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse *et al.*, *Nuc. Acids. Res.* 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

30       The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (Cabilly *et al.*, *supra*; Morrison, *et al.*, *Proc. Nat. Acad. Sci. USA* 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an  
35       antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using

a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

### 3. Humanized and human antibodies

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-327 (1988); Verhoeven *et al.*, *Science* 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly *et al.*, *supra*), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims *et al.*, *J. Immunol.* 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.* 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA* 89:4285 (1992); Presta *et al.*, *J. Immunol.* 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region ( $J_H$ ) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody

production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90:2551 (1993); Jakobovits *et al.*, *Nature* 362:255-258 (1993); Bruggermann *et al.*, *Year in Immuno.* 7:33 (1993). Human antibodies can also be produced in phage- display libraries (Hoogenboom *et al.*,  
5 *J. Mol. Biol.* 227:381 (1991); Marks *et al.*, *J. Mol. Biol.* 222:581 (1991)).

#### 4. Bispecific antibodies

Bispecific antibodies (BsAbs) are antibodies that have binding specificities for at least two different antigens. BsAbs can be used as tumor targeting or imaging agents and can be used to target enzymes or toxins to a cell possessing the WSX receptor. Such antibodies can be derived from full length antibodies or antibody  
10 fragments (e.g. F(ab')<sub>2</sub> bispecific antibodies). In accordance with the present invention, the BsAb may possess one arm which binds the WSX receptor and another arm which binds to a cytokine or another cytokine receptor (or a subunit thereof) such as the receptors for TPO, EPO, G-CSF, IL-4, IL-7, GH, PRL; the  $\alpha$  or  $\beta$  subunits of the IL-3, GM-CSF, IL-5, IL-6, LIF, OSM and CNTF receptors; or the  $\alpha$ ,  $\beta$  or  $\gamma$  subunits of the IL-2 receptor complex. For example, the BsAb may bind both WSX receptor and gp130.

15 Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein *et al.*, *Nature* 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the  
20 correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, *EMBO J.* 10:3655-3659 (1991).

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences.  
25 The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual  
30 proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid  
35 immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690 published March 3, 1994.

For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology* 121:210 (1986).

Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in US Patent No. 4,676,980, along with a number of cross-linking techniques.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. The following techniques can also be used for the production of bivalent antibody fragments which are not necessarily bispecific. According to these techniques, Fab'-SH fragments can be recovered from *E. coli*, which can be chemically coupled to form bivalent antibodies. Shalaby *et al.*, *J. Exp. Med.* 175:217-225 (1992) describe the production of a fully humanized BsAb F(ab')<sub>2</sub> molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the BsAb. The BsAb thus formed was able to bind to cells overexpressing the HER2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets. See also Rodrigues *et al.*, *Int. J. Cancers* (Suppl.) 7:45-50 (1992).

Various techniques for making and isolating bivalent antibody fragments directly from recombinant cell culture have also been described. For example, bivalent heterodimers have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.* 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993) has provided an alternative mechanism for making BsAb fragments. The fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V<sub>H</sub> and V<sub>L</sub> domains of one fragment are forced to pair with the complementary V<sub>L</sub> and V<sub>H</sub> domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making BsAb fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber *et al.*, *J. Immunol.* 152:5368 (1994).

### 30 5. Antibody Screening

It may be desirable to select antibodies with a strong binding affinity for the WSX receptor. Antibody affinities may be determined by saturation binding; enzyme-linked immunoabsorbent assay (ELISA); and competition assays (e.g. RIA's), for example. The antibody with a strong binding affinity may bind the WSX receptor with a binding affinity (K<sub>d</sub>) value of no more than about  $1 \times 10^{-7}$  M, preferably no more than about  $1 \times 10^{-8}$  M and most preferably no more than about  $1 \times 10^{-9}$  M (e.g. to about  $1 \times 10^{-12}$  M).

In another embodiment, one may screen for an antibody which binds a WSX receptor epitope of interest. For example, an antibody which binds to the epitope bound by antibody 2D7, 1G4, 1E11 or 1C11 (see Example 13) or antibody clone #3, #4 or #17 (see Example 14) can be identified. To screen for antibodies which bind to the epitope on WSX receptor bound by an antibody of interest (e.g., those which block binding of any one

of the above antibodies to WSX receptor), a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. Alternatively, epitope mapping, *e.g.* as described in Champe *et al.*, *J. Biol. Chem.* 270:1388-1394 (1995), can be performed to determine whether the antibody binds an epitope of interest.

5 In one particularly preferred embodiment of the invention, agonist antibodies are selected. Various methods for selecting agonist antibodies are available. In one embodiment, one evaluates the agonistic properties of the antibody upon binding to a chimeric receptor comprising the WSX receptor extracellular domain in an assay called the kinase receptor activation enzyme linked immunoadsorbent assay (KIRA ELISA) described in WO95/14930 (expressly incorporated herein by reference).

10 To perform the KIRA ELISA, a chimeric receptor comprising the extracellular domain of the WSX receptor and the transmembrane and intracellular domain of Rse receptor (Mark *et al.*, *Journal of Biological Chemistry* 269(14):10720-10728 (1994)) with a carboxyl-terminal herpes simplex virus glycoprotein D (gD) tag is produced and dp12.CHO cells are transformed therewith as described in Example 4 of WO95/14930.

The WSX/Rse.gD transformed dp12.CHO cells are seeded ( $3 \times 10^4$  per well) in the wells of a flat-bottom-96 well culture plate in 100  $\mu$ l media and cultured overnight at 37°C in 5% CO<sub>2</sub>. The following morning the well supernatants are removed and various concentrations of the antibody are added to separate wells. The cells are stimulated at 37°C for 30 min., the well supernatants are decanted. To lyse the cells and solubilize the chimeric receptors, 100  $\mu$ l of lysis buffer is added to each well. The plate is then agitated gently on a plate shaker (Bellco Instruments, Vineland, NJ) for 60 min. at room temperature.

20 While the cells are being solubilized, an ELISA microtiter plate (Nunc Maxisorp, Inter Med, Denmark) coated overnight at 4°C with the 5B6 monoclonal anti-gD antibody (5.0  $\mu$ g/ml in 50 mM carbonate buffer, pH 9.6, 100  $\mu$ l/well) is decanted and blocked with 150  $\mu$ l/well of Block Buffer for 60 min. at room temperature. After 60 minutes, the anti-gD 5B6 coated plate is washed 6 times with wash buffer (PBS containing 0.05 % TWEEN 20™ and 0.01 % thimerosal).

25 The lysate containing solubilized WSX/Rse.gD from the cell-culture microtiter well is transferred (85  $\mu$ l/well) to anti-gD 5B6 coated and blocked ELISA well and is incubated for 2 h at room temperature. The unbound WSX/Rse.gD is removed by washing with wash buffer and 100  $\mu$ l of biotinylated 4G10 (anti-phosphotyrosine) diluted 1:18000 in dilution buffer (PBS containing 0.5 % BSA, 0.05 % Tween-20, 5 mM EDTA, and 0.01 % thimerosal), *i.e.* 56 ng/ml is added to each well. After incubation for 2 h at room temperature the plate is washed and HRPO-conjugated streptavidin (Zymed Laboratories, S. San Francisco, CA) is added to each well. The plate is incubated for 30 minutes at room temperature with gentle agitation. The free avidin-conjugate is washed away and 100  $\mu$ l freshly prepared substrate solution (tetramethyl benzidine (TMB); 2-component substrate kit; Kirkegaard and Perry, Gaithersburg, MD) is added to each well. The reaction is allowed to proceed for 10 minutes, after which the color development is stopped by the addition of 100  $\mu$ l/well  
35 1.0 M H<sub>3</sub>PO<sub>4</sub>. The absorbance at 450 nm is read with a reference wavelength of 650 nm (ABS<sub>450/650</sub>), using a *vmax* plate reader (Molecular Devices, Palo Alto, CA) controlled with a Macintosh Centris 650 (Apple Computers, Cupertino, CA) and DeltaSoft software (BioMetallics, Inc, Princeton, NJ).

Those antibodies which have an IC<sub>50</sub> in the KIRA ELISA of about 0.5 µg/ml or less (e.g. from about 0.5 µg/ml to about 0.001 µg/ml), preferably about 0.2 µg/ml or less and most preferably about 0.1 µg/ml or less are preferred agonists.

In another embodiment, one screens for antibodies which activate downstream signaling molecules for OB protein. For example, the ability of the antibody to activate Signal Transducers and Activators of Transcription (STATs) can be assessed. The agonist antibody of interest may stimulate formation of STAT-1 and STAT-3 complexes, for example. To screen for such antibodies, the assay described in Rosenblum *et al. Endocrinology* 137(11):5178-5181 (1996) may be performed.

Alternatively, an antibody which stimulates proliferation and/or differentiation of hematopoietic cells can be selected. For example, the hematopoiesis assays of Example 10 below can be performed. For example, murine fetal liver fLASK stem cells may be isolated from the midgestational fetal liver as described in Zeigler *et al., Blood* 84:2422-2430 (1994) and studied in stem cell suspension culture or methylcellulose assays. For the stem cell suspension cultures, twenty thousand of the fLASK cells are seeded in individual wells in a 12 well format in DMEM 4.5/F12 media supplemented with 10% heat inactivated fetal calf serum (Hyclone, Logan, UT) and L-glutamine. Growth factors are added at the following concentrations: kit ligand (KL) at 25 ng/mL, interleukin-3 (IL-3) at 25 ng/mL, interleukin-6 (IL-6) at 50 ng/mL, G-CSF at 100 ng/mL, GM-CSF at 100 ng/mL, EPO at 2U/mL, interleukin-7 (IL-7) at 100 ng/mL (all growth factors from R and D Systems, Minneapolis, MN). The agonist antibody is then added and the ability of the antibody to expand the fLASK cells grown in suspension culture is assessed. Methylcellulose assays are performed as previously described (Zeiger *et al., supra*). Briefly, methylcellulose colony assays are performed using "complete" methylcellulose or pre-B methylcellulose medium (Stem Cell Technologies, Vancouver, British Columbia, Canada) with the addition of 25 ng/mL KL (R and D Systems, Minneapolis, MN). Cytospin analyses of the resultant colonies are performed as previously described in Zeigler *et al.* The ability of the agonist antibody to augment myeloid, lymphoid and erythroid colony formation is assessed. Also, the effect of the agonist antibody on the murine bone marrow stem cell population; Lin<sup>lo</sup>Sca<sup>+</sup> may be evaluated.

One may select an agonist antibody which induces a statistically significant decrease in body weight and/or fat-depot weight and/or food intake in an obese mammal (e.g. in an *ob/ob* mouse). Methods for screening for such molecules are described in Levin *et al. Proc. Natl. Acad. Sci. USA* 93:1726-1730 (1996), for example. Preferred agonist antibodies are those which exert adipose-reducing effects in an obese mammal, such as the *ob/ob* mouse, which are in excess of those induced by reductions in food intake.

The antibody of interest herein may have the hypervariable region residues of one of the antibodies in Examples 13 and 14. Also, the invention encompasses "affinity matured" forms of these antibodies in which hypervariable region residues of these antibodies have been modified. Such affinity matured antibodies will preferably have a biological activity which is the same as or better than that of the original antibody. The affinity matured antibody may have from about 1-10, e.g. 5-10 deletions, insertions or substitutions (but preferably substitutions) in the hypervariable regions thereof. One useful procedure for generating affinity matured antibodies is called "alanine scanning mutagenesis" (Cunningham and Wells *Science* 244:1081-1085 (1989)). Here, one or more of the hypervariable region residue(s) are replaced by alanine or polyalanine residue(s) to affect the interaction of the amino acids with the WSX receptor. Those hypervariable region residue(s)

demonstrating functional sensitivity to substitution are then refined by introducing further or other mutations at or for the sites of substitution. The ala-mutants produced this way are screened for their biological activity as described herein. Another procedure is affinity maturation using phage display (Hawkins *et al.* *J. Mol. Biol.* 254:889-896 (1992) and Lowman *et al.* *Biochemistry* 30(45):10832-10837 (1991)). Briefly, several  
5 hypervariable region sites (*e.g.* 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody mutants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed mutants are then screened for their biological activity (*e.g.* binding affinity).

#### 6. Antibody Modifications

10 It may be desirable to tailor the antibody for various applications. Exemplary antibody modifications are described here.

In certain embodiments of the invention, it may be desirable to use an antibody fragment, rather than an intact antibody. In this case, it may be desirable to modify the antibody fragment in order to increase its serum half-life. This may be achieved, for example, by incorporation of a salvage receptor binding epitope into the  
15 antibody fragment. See WO96/32478 published October 17, 1996. Alternatively, the antibody may be conjugated to a nonproteinaceous polymer, such as those described above for the production of long half-life derivatives of OB protein.

Where the antibody is to be used to treat cancer for example, various modifications of the antibody (*e.g.* of a neutralizing antibody) which enhance the effectiveness of the antibody for treating cancer are contemplated  
20 herein. For example, it may be desirable to modify the antibody of the invention with respect to effector function. For example cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp. Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.*  
25 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* *Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.* *Anti-Cancer Drug Design* 3:219-230 (1989).

The invention also pertains to immunoconjugates comprising the antibody described herein conjugated  
30 to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.* an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. A variety  
35 of radionuclides are available for the production of radioconjugate antibodies. Examples include <sup>212</sup>Bi, <sup>131</sup>I, <sup>131</sup>In, <sup>90</sup>Y and <sup>186</sup>Re.



Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al. Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

10 In another embodiment, the antibody may be conjugated to a "receptor" (such streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide).

The antibody may also be formulated as an immunoliposome. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al., Proc. Natl. Acad. Sci. USA*, 82:3688 (1985); Hwang *et al., Proc. Natl. Acad. Sci. USA*, 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al. J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as Doxorubicin) is optionally contained within the liposome. See Gabizon *et al. J. National Cancer Inst.* 81(19):1484 (1989).

25 The antibody of the present invention may also be used in ADEPT by conjugating the antibody to a prodrug-activating enzyme which converts a prodrug (e.g. a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278.

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active, cytotoxic form.

30 Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as  $\beta$ -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs;  $\beta$ -lactamase useful for converting drugs derivatized with  $\beta$ -lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively,

into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, e.g., Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

5           The enzymes of this invention can be covalently bound to the antibody mutant by techniques well known in the art such as the use of the heterobifunctional crosslinking reagents discussed above. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, e.g., Neuberger *et al.*, *Nature*, 312: 604-608 (1984)).

10           In other embodiments, the antibody can be covalently modified, with exemplary such modifications described above.

#### **E.       Therapeutic Uses for WSX Receptor Ligands and Antibodies**

          The WSX ligands (e.g. OB protein and anti-WSX receptor agonist antibodies) of the present invention are useful, in one embodiment, for weight reduction, and specifically, in the treatment of obesity, bulimia and  
15   other disorders associated with the abnormal expression or function of the OB and/or WSX receptor genes, other metabolic disorders such as diabetes, for reducing excessive levels of insulin in human patients (e.g. to restore or improve the insulin-sensitivity of such patients). Thus, these molecules can be used to treat a patient suffering from excessive food consumption and related pathological conditions such as type II adult onset diabetes, infertility (Chehab *et al.* *Nature Genetics* 12:318-320 (1996)), hypercholesterolemia, hyperlipidemia,  
20   cardiovascular diseases, arteriosclerosis, polycystic ovarian disease, osteoarthritis, dermatological disorders, insulin resistance, hypertriglyceridemia, cancer, cholelithiasis and hypertension.

          In addition, the WSX ligands can be used for the treatment of kidney ailments, hypertension, and lung dysfunctions, such as emphysema.

          In a further embodiment, the WSX ligands (such as agonist WSX receptor antibodies) of the present  
25   invention can be used to enhance repopulation of mature blood cell lineages in mammals having undergone chemo- or radiation therapy or bone marrow transplantation therapy. Generally, the ligands will act via an enhancement of the proliferation and/or differentiation (but especially proliferation) of primitive hematopoietic cells. The ligands may similarly be useful for treating diseases characterized by a decrease in blood cells. Examples of these diseases include: anemia (including macrocytic and aplastic anemia); thrombocytopenia;  
30   hypoplasia; immune (autoimmune) thrombocytopenic purpura (ITP); and HIV induced ITP. Also, the ligands may be used to treat a patient having suffered a hemorrhage. WSX ligands may also be used to treat metabolic disorders such as obesity and diabetes mellitus, or to promote kidney, liver or lung growth and/or repair (e.g., in renal failure).

          The WSX receptor ligands and antibodies may be administered alone or in concert with one or more  
35   cytokines. Furthermore, as an alternative to administration of the WSX ligand protein, gene therapy techniques (discussed in the section above entitled "Therapeutic Uses for the WSX Receptor") are also contemplated herein.

          Potential therapeutic applications for WSX receptor neutralizing antibodies include the treatment of metabolic disorders (such as cachexia, anorexia and other wasting diseases characterized by loss of appetite,

diminished food intake or body weight loss), stem cell tumors and other tumors at sites of WSX receptor expression, especially those tumors characterized by overexpression of WSX receptor.

For therapeutic applications, the WSX receptor ligands and antibodies of the invention are administered to a mammal, preferably a human, in a physiologically acceptable dosage form, including those that may be administered to a human intravenously as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intra-cerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The WSX receptor ligands and antibodies also are suitably administered by intratumoral, peritumoral, intralesional, or perilesional routes or to the lymph, to exert local as well as systemic therapeutic effects.

Such dosage forms encompass physiologically acceptable carriers that are inherently non-toxic and non-therapeutic. Examples of such carriers include ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts, or electrolytes such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, and PEG. Carriers for topical or gel-based forms of WSX receptor antibodies include polysaccharides such as sodium carboxymethylcellulose or methylcellulose, polyvinylpyrrolidone, polyacrylates, polyoxyethylene-polyoxypropylene-block polymers, PEG, and wood wax alcohols. For all administrations, conventional depot forms are suitably used. Such forms include, for example, microcapsules, nano-capsules, liposomes, plasters, inhalation forms, nose sprays, sublingual tablets, and sustained-release preparations. The WSX receptor ligand or antibody will typically be formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml.

Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the WSX receptor ligand or antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate) as described by Langer *et al.*, *supra* and Langer, *supra*, or poly(vinylalcohol), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$  ethyl-L-glutamate (Sidman *et al.*, *supra*), non-degradable ethylene-vinyl acetate (Langer *et al.*, *supra*), degradable lactic acid-glycolic acid copolymers such as the Lupron Depot<sup>TM</sup> (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated WSX receptor antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

Sustained-release WSX receptor ligand or antibody compositions also include liposomally entrapped antibodies. Liposomes containing the WSX receptor ligand or antibody are prepared by methods known in the

art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA* 82:3688 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Ordinarily, the liposomes are the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol.% cholesterol, the selected proportion being adjusted for the optimal WSX receptor ligand or antibody therapy.

5 Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

For the prevention or treatment of disease, the appropriate dosage of WSX receptor ligand or antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibodies are administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the WSX receptor ligand or antibody, and the discretion of the attending physician. The

10 WSX receptor ligand or antibody is suitably administered to the patient at one time or over a series of treatments.

Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg of WSX receptor ligand or antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to 100 µg/kg (*e.g.* 1-50 µg/kg) or more, depending on the factors mentioned above. For example, the dose may be

15 the same as that for other cytokines such as G-CSF, GM-CSF and EPO. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

When one or more cytokines are co-administered with the WSX receptor ligand, lesser doses of the

20 WSX ligand may be employed. Suitable doses of a cytokine are from about 1 µg/kg to about 15mg/kg of cytokine. A typical daily dosage of the cytokine might range from about 1 µg/kg to 100 µg/kg (*e.g.* 1-50 µg/kg) or more. For example, the dose may be the same as that for other cytokines such as G-CSF, GM-CSF and EPO. The cytokine(s) may be administered prior to, simultaneously with, or following administration of the WSX ligand. The cytokine(s) and WSX ligand may be combined to form a pharmaceutically composition for

25 simultaneous administration to the mammal. In certain embodiments, the amounts of WSX ligand and cytokine are such that a synergistic repopulation of blood cells (or synergistic increase in proliferation and/or differentiation of hematopoietic cells) occurs in the mammal upon administration of the WSX ligand and cytokine thereto. In other words, the coordinated action of the two or more agents (*i.e.* the WSX ligand and cytokine(s)) with respect to repopulation of blood cells (or proliferation/differentiation of hematopoietic cells)

30 is greater than the sum of the individual effects of these molecules.

For treating obesity and associated pathological conditions, the WSX ligand may be administered in combination with other treatments for combatting or preventing obesity. Substances useful for this purpose include, *e.g.*, hormones (catecholamines, glucagon, ACTH); clofibrate; halogenate; cinchocaine; chlorpromazine; appetite-suppressing drugs acting on noradrenergic neurotransmitters such as mazindol and derivatives of

35 phenethylamine, *e.g.*, phenylpropanolamine, diethylpropion, phentermine, phendimetrazine, benzphetamine, amphetamine, methamphetamine, and phenmetrazine; drugs acting on serotonin neurotransmitters such as fenfluramine, tryptophan, 5-hydroxytryptophan, fluoxetine, and sertraline; centrally active drugs such as naloxone, neuropeptide-Y, galanin, corticotropin-releasing hormone, and cholecystokinin; a cholinergic agonist such as pyridostigmine; a sphingolipid such as a lysosphingolipid or derivative thereof (EP 321,287 published

June 21, 1989); thermogenic drugs such as thyroid hormone, ephedrine, beta-adrenergic agonists; drugs affecting the gastrointestinal tract such as enzyme inhibitors, *e.g.*, tetrahydrolipostatin, indigestible food such as sucrose polyester, and inhibitors of gastric emptying such as threo-chlorocitric acid or its derivatives;  $\beta$ -adrenergic agonist such as isoproterenol and yohimbine; aminophylline to increase the  $\beta$ -adrenergic-like effects of yohimbine, an  $\alpha_2$ -adrenergic blocking drug such as clonidine alone or in combination with a growth hormone releasing peptide (U.S. Pat. No. 5,120,713 issued June 9, 1992); drugs that interfere with intestinal absorption such as biguanides such as metformin and phenformin; bulk fillers such as methylcellulose; metabolic blocking drugs such as hydroxycitrate; progesterone; cholecystokinin agonists; small molecules that mimic ketoacids; agonists to corticotropin-releasing hormone; an ergot-related prolactin-inhibiting compound for reducing body fat stores (U.S. Pat. No. 4,783,469 issued November 8, 1988); beta-3-agonists; bromocriptine; antagonists to opioid peptides; antagonists to neuropeptide Y; glucocorticoid receptor antagonists; growth hormone agonists; combinations thereof; *etc.* This includes all drugs described by Bray and Greenway, *Clinics in Endocrinol. and Metabol.*, 5:455 (1976).

These adjunctive agents may be administered at the same time as, before, or after the administration of WSX ligand and can be administered by the same or a different administration route than the WSX ligand.

The WSX ligand treatment may occur without, or may be imposed with, a dietary restriction such as a limit in daily food or calorie intake, as is desired for the individual patient.

#### F. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the conditions described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is the WSX ligand. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container holding a cytokine for co-administration with the WSX ligand. Further container(s) may be provided with the article of manufacture which may hold, for example, a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution or dextrose solution. The article of manufacture may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

#### G. Non-Therapeutic Uses for WSX Receptor Ligands and Antibodies

WSX receptor ligands and antibodies may be used for detection of and/or enrichment of hematopoietic stem cell/progenitor cell populations in a similar manner to that in which CD34 antibodies are presently used. For stem cell enrichment, the WSX receptor antibodies may be utilized in the techniques known in the art such as immune panning, flow cytometry or immunomagnetic beads.

In accordance with one *in vitro* application of the WSX ligands, cells comprising the WSX receptor are provided and placed in a cell culture medium. Examples of such WSX-receptor-containing cells include hematopoietic progenitor cells, such as CD34+ cells.

Suitable tissue culture media are well known to persons skilled in the art and include, but are not limited to, Minimal Essential Medium (MEM), RPMI-1640, and Dulbecco's Modified Eagle's Medium (DMEM). These tissue culture medias are commercially available from Sigma Chemical Company (St. Louis, MO) and GIBCO (Grand Island, NY). The cells are then cultured in the cell culture medium under conditions sufficient for the cells to remain viable and grow in the presence of an effective amount of WSX ligand and, optionally, further cytokines and growth factors. The cells can be cultured in a variety of ways, including culturing in a clot, agar, or liquid culture.

The cells are cultured at a physiologically acceptable temperature such as 37° C, for example, in the presence of an effective amount of WSX ligand. The amount of WSX ligand may vary, but preferably is in the range of about 10 ng/ml to about 1mg/ml. The WSX ligand can of course be added to the culture at a dose determined empirically by those in the art without undue experimentation. The concentration of WSX ligand in the culture will depend on various factors, such as the conditions under which the cells and WSX ligand are cultured. The specific temperature and duration of incubation, as well as other culture conditions, can be varied depending on such factors as, *e.g.*, the concentration of the WSX ligand, and the type of cells and medium.

It is contemplated that using WSX ligand to enhance cell proliferation and/or differentiation *in vitro* will be useful in a variety of ways. For instance, hematopoietic cells cultured *in vitro* in the presence of WSX ligand can be infused into a mammal suffering from reduced levels of the cells. Also, the cultured hematopoietic cells may be used for gene transfer for gene therapy applications. Stable *in vitro* cultures can be also used for isolating cell-specific factors and for expression of endogenous or recombinantly introduced proteins in the cell. WSX ligand may also be used to enhance cell survival, proliferation and/or differentiation of cells which support the growth and/or differentiation of other cells in cell culture.

The WSX receptor antibodies of the invention are also useful as affinity purification agents. In this process, the antibodies against WSX receptor are immobilized on a suitable support, such a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the WSX receptor to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the WSX receptor, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent, such as glycine buffer, pH 5.0, that will release the WSX receptor from the antibody.

WSX receptor antibodies may also be useful in diagnostic assays for WSX receptor, *e.g.*, detecting its expression in specific cells, tissues, or serum. For diagnostic applications, antibodies typically will be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as <sup>3</sup>H, <sup>14</sup>C, <sup>32</sup>P, <sup>35</sup>S, or <sup>125</sup>I; a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; radioactive isotopic labels, such as, *e.g.*, <sup>125</sup>I, <sup>32</sup>P, <sup>14</sup>C, or <sup>3</sup>H; or an enzyme, such as alkaline phosphatase, beta-galactosidase, or horseradish peroxidase.

Any method known in the art for separately conjugating the polypeptide variant to the detectable moiety may be employed, including those methods described by Hunter *et al.*, *Nature* 144:945 (1962); David *et al.*, *Biochemistry* 13:1014 (1974); Pain *et al.*, *J. Immunol. Meth* 40:219 (1981); and Nygren, *J. Histochem. and Cytochem.* 30:407 (1982).

The antibodies of the present invention may be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc., 1987).

Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of WSX receptor in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, e.g., US Pat No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

#### H. Deposit of Materials

The following biological materials have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC):

20	Deposit Designation	ATCC No.	Deposit Date
	Baf3/WSX E63x7 sort (Baf3 cells expressing human WSX receptor variant 13.2)	ATCC CRL 12015	Jan 10, 1996
	2D7 hybridoma cell line		
25	1G4 hybridoma cell line	ATCC HB-12243	Dec 11, 1996
	1E11 hybridoma cell line		
	1C11 hybridoma cell line		

These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture for 30 years from the date of deposit. Each of the deposited cultures will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures (a) that access to the culture will be available during pendency of the patent application to one determined by the Commissioner to be entitled thereto under

37 CFR §1.14 and 35 USC §122, and (b) that all restrictions on the availability to the public of the culture so deposited will be irrevocably removed upon the granting of the patent.

The assignee of the present application has agreed that if any of the cultures on deposit should die or be lost or destroyed when cultivated under suitable conditions, it will be promptly replaced on notification with a viable specimen of the same culture. Availability of the deposited cell lines is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by any culture deposited, since the deposited embodiment is intended as an illustration of one aspect of the invention and any culture that is functionally equivalent is within the scope of this invention. The deposit of material herein does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustration that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

### III. Experimental

Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

The disclosures of all publications, patents and patent applications cited herein, whether *supra* or *infra*, are hereby incorporated by reference in their entirety.

#### EXAMPLE 1

##### Cloning of Human WSX Receptor

An oligonucleotide probe designated WSX.6 #1 was synthesized based upon the T73849 EST sequence.

The WSX.6 #1 probe was a 51mer having the following sequence:

5' GTCAGTCTCCAGTTCAGACTTGTGTGCAGTCTATGCTGTTTCAGGTGCGC - 3' (SEQ ID NO:45).

The radiolabeled WSX.6 #1 probe was used to probe  $1.2 \times 10^6$  clones from a random and oligo dT primed  $\lambda$ gt10 fetal liver library (Clontech, Palo Alto, CA). Following hybridization at 42°C overnight, the filters were washed at 50°C in 0.5 x SSC and 0.1% NaDodSO<sub>4</sub> (SDS). From the initial screen, 10 clones were selected and upon subsequent screening 5 individual plaque pure clones were isolated. Of these 5 individual clones, four clones designated 1, 5, 6 and 9 were subcloned into pBSSK<sup>+</sup> (Stratagene) following EcoRI digestion. Sequence analysis revealed clone 5 and clone 9 contained the putative initiation methionine and signal peptide. Clone 6 (designated 6.4) contained the most 3' end sequence and subsequently was used for further screening.

To obtain the full length gene, clone 6.4 (fragment Nsi-Hind III) was radiolabeled and used to screen  $1.2 \times 10^6$  clones from a  $\lambda$ gt 10 library constructed from a hepatoma Hep3B cell line. This screen resulted in 24 positive clones. Following PCR analysis of the clones using  $\lambda$ gt10 primers (F and R), the four longest clones 12.1, 13.2, 22.3, and 24.3 were isolated. These clones were subcloned into pBSSK<sup>+</sup> using the EcoRI site, and following examination by restriction enzyme digest, clones 12.1 and 13.2 were submitted for sequencing. DNA sequencing was performed with the Taq dye deoxynucleotide terminator cycle sequencing kit on an automated Applied Biosystems DNA sequencer.



The assembled contiguous sequence from all the isolated clones encoded a consensus amino terminus for the newly identified polypeptide designated the WSX receptor. However, sequence analysis revealed that at least three naturally occurring variants of the WSX receptor exist which have different cytoplasmic regions. These variants appear to be differentially spliced at the lysine residue at position 891. Clone 6.4 stops 5 amino acids after Lys 891. Clone 12.1 is different from 13.2 and 6.4 following Lys 891 and encodes a putative box 2 region which is distinct from that encoded by clone 13.2. Clone 13.2 contains a potential box 1 region and following Lys 891 encodes putative box 2 and box 3 motifs. See, Baumann *et al.*, *Mol. Cell. Biol.* 14(1):138-146 (1994).

The full length WSX gene based on the clone 13.2 cytoplasmic region putatively encodes an 1165 amino acid transmembrane protein. The 841 amino acid extracellular domain (ECD) contains two WSXWS domains. The ECD is followed by a 24 amino acid transmembrane domain and a 300 amino acid cytoplasmic region.

## EXAMPLE 2

### WSX Receptor Immunoadhesin

Using polymerase chain amplification, a WSX receptor immunoadhesin was created by engineering an in-frame fusion of the WSX receptor gene extracellular domain (WSX.ECD) with human CH<sub>2</sub>CH<sub>3</sub>(Fc)IgG (Bennett *et al.*, *J.Biol. Chem.* 266(34):23060-23067 (1991)) at the C terminus of the ECD and cloned into pBSSK<sup>+</sup> (Stratagene). For expression, the WSX-Fc was excised with ClaI and BstEII and ligated into the pRK5.HuIF.grb1IgG Genenase I vector (Beck *et al.*, *Molecular Immunology* 31(17):1335-1344 (1994)), to create the plasmid pRK5.WSX-IgG Genenase I. This plasmid was transiently transfected into 293 cells using standard calcium phosphate transfection techniques. The transfected cells were cultured at 37°C in 5% CO<sub>2</sub> in DMEM F12 50:50 supplemented with 10% FBS, 100mM HEPES (pH 7.2) and 1mM glutamine. The WSX receptor immunoadhesin was purified using a ProSepA<sup>TM</sup> protein A column.

## EXAMPLE 3

### Antibody Production

In order to raise antibodies against the WSX receptor, the WSX receptor immunoadhesin of Example 2 was used to inoculate rabbits to raise polyclonal antibodies and mice to raise monoclonal antibodies using conventional technology.

## EXAMPLE 4

### Generation of a Cell Line Expressing WSX Receptor

The nucleic acid encoding full length WSX receptor variant 13.2 was inserted in the pRKtkNeo plasmid (Holmes *et al.*, *Science* 253:1278-1280 (1991)). 100 µg of the pRKtkNeo.WSX plasmid thus generated was linearized, ethanol precipitated and resuspended in 100 µL of RPMI 1640. 7 x 10<sup>6</sup> Baf3 cells (5 x 10<sup>5</sup>/ml) were suspended in 900 µL of RPMI and added to the linearized plasmid. Following electroporation at 325V, 1180 µF using a BRL electroporation apparatus, the cells were plated into 15 mls of RPMI 1640 containing 5% WEHI3B conditioned media and 15% serum. 48 hours later cells were selected in 2mg/ml G418.

To obtain the Baf3/WSX cell line expressing WSX receptor variant 13.2, the G418 selected clones were analyzed by FACS using the rabbit polyclonal antisera raised against the WSX-Fc chimeric protein as described above. The highest expressing clone (designated E6) was sorted by FACS to maintain a population with a high level of WSX receptor expression.

**EXAMPLE 5****Role of WSX Receptor in Cellular Proliferation**

The proliferative potentials of WSX receptor variants 13.2 and 12.1 were tested by constructing human growth hormone receptor-WSX receptor (GH-WSX) fusions encoding chimeric proteins consisting of the GH receptor extracellular and transmembrane domains and the WSX receptor variant 13.2 or 12.1 intracellular domains. These chimeric gene fusions were transfected into the IL-3 dependent cell line Baf3. The ability of the GH-WSX transfected Baf3 cells to respond to exogenous growth hormone (GH) was tested in a thymidine incorporation assay. As can be seen in Figs. 6 and 8, the GH-WSX receptor variant 13.2 chimera was capable of increasing thymidine uptake in the transfected Baf3 cells, thus indicating the proliferative potential of the WSX receptor variant 13.2. However, WSX receptor variant 12.1 was unable to transmit a proliferative signal in this experiment (Fig. 8).

**Materials and Methods**

Recombinant PCR was used to generate the chimeric receptors containing the extracellular and transmembrane domains of the hGH receptor and the cytoplasmic domain of either WSX receptor variant 12.1 or variant 13.2. In short, the cytoplasmic domain of either variant 12.1 or 13.2 beginning with Arg at amino acid 866 and extending down to amino acid 958 or amino acid 1165 respectively, was fused in frame, by sequential PCR, to the hGH receptor extracellular and transmembrane domain beginning with Met at amino acid 18 and extending down to Arg at amino acid 274. The GH-WSX chimera was constructed by first using PCR to generate the extracellular and transmembrane domain of the human GH receptor. The 3' end primer used for this PCR contained 20 nucleotides at the 5' end of the primer corresponding to the first 20 nucleotides of the WSX cytoplasmic domain. The 3' end of the chimera was generated using PCR where the 5' end primer contained the last 19 nucleotides of the human GH receptor transmembrane domain. To generate the full length chimera, the 5' end of the human GH receptor product was combined with the 3' end WSX receptor cytoplasmic PCR product and subsequently amplified to create a fusion of the two products.

This chimeric fusion was digested with ClaI and XbaI and ligated to pRKtkNeo (Holmes *et al.*, *Science* 253:1278-1280 (1991)) to create the chimeric expression vector. The IL-3 dependent cell line Baf3 was then electroporated with this hGH/WSX chimeric expression vector.

Briefly, 100 $\mu$ g of the pRKtkNeo/GH.WSX plasmid was linearized, ethanol precipitated and resuspended in 100  $\mu$ L of RPMI 1640.  $7 \times 10^6$  Baf3 cells ( $5 \times 10^5$ /ml) were suspended in 900  $\mu$ L of RPMI and added to the linearized plasmid. Following electroporation at 325V, 1180  $\mu$ F using a BRL electroporation apparatus, the cells were plated into 15 mls of RPMI 1640 containing 5% wehi conditioned media and 15% serum. 48 hours later, cells were selected in 2mg/ml G418.

To obtain the Baf3/GH.WSX cell lines, the G418 selected cells were FACS sorted using an anti-human GH mAb (3B7) at 1 $\mu$ g/ml. The top 10% expressing cells were selected and expanded.

**EXAMPLE 6****Expression Analysis of the WSX Receptor**

The expression profile of the WSX receptor was initially examined by Northern analysis. Northern blots of human fetal or adult tissue mRNA were obtained from Clontech (Palo Alto, California). A transcript of approximately 6 kb was detected in human fetal lung, liver and kidney. In the adult, low level expression was

detected in a variety of tissues including liver, placenta, lung skeletal muscle, kidney, ovary, prostate and small intestine.

PCR analysis of human cord blood identified transcripts in CD34<sup>+</sup> subfraction. By PCR analysis, all three variants of the WSX receptor were present in CD34<sup>+</sup> cells. The CD34<sup>-</sup> subfraction appeared negative by this same PCR analysis.

By PCR analysis, both the 6.4 variant and 13.2 variant were evident in the AA4<sup>+</sup>Sca<sup>+</sup>Kit<sup>+</sup> (fASK) cell population isolated from the mid-gestation fetal liver as described in Zeigler *et al.*, *Blood* 84:2422-2430 (1994). No clones containing the 12.1 variant cytoplasmic tail have been isolated from murine tissues.

Human B cells isolated from peripheral blood using anti-CD19/20 antibodies were also positive for short form (6.4 variant) and long form (13.2 variant) receptor mRNA expression.

The WSX receptor appears to be expressed on both progenitor and more mature hematopoietic cells.

#### EXAMPLE 7

##### Cloning of Murine WSX Receptor

The human WSX receptor was used as a probe to isolate murine WSX receptor. The pRKtkNeo.WSX plasmid of Example 4 was digested using SspI. This SspI fragment (1624 bps) was isolated, and radiolabelled, and used to screen a murine liver  $\lambda$ gt10 library (Clontech). This resulted in 4 positive clones which were isolated and sequenced after sub-cloning into pBSSK<sup>+</sup> via EcoRI digestion. The resultant clones, designated 1, 2, 3, 4 showed homology to the extracellular domain of the human WSX receptor; the contiguous sequences resulting from these clones extended from the initiation methionine to tryptophan at position 783. The overall similarity of human WSX receptor and murine WSX receptor is 73 % over this region of the respective extracellular domains (see Figs. 4A-B).

#### EXAMPLE 8

##### The Role of WSX Receptor in Hematopoietic Cell Proliferation

The presence of the WSX receptor in the enriched human stem cell population CD34<sup>+</sup> from cord blood is indicative of a potential role for this receptor in stem cell/progenitor cell proliferation. The proliferation of CD34<sup>+</sup> human blood cells in methylcellulose media (Stem Cell Technologies) was determined in the presence or absence of WSX receptor antisense oligonucleotides. These experiments were also repeated in the murine hematopoietic system using AA4<sup>+</sup> Sca<sup>+</sup> Kit<sup>+</sup> stem cells from the murine fetal liver. In both instances, the antisense oligonucleotides statistically significantly inhibited colony formation from the hematopoietic progenitor cells. See Table 1 below. The anti-proliferative effects were most pronounced using the -20 antisense and the +85 antisense oligonucleotide constructs. This inhibition was not lineage specific to any particular myeloid lineage that resulted from the progenitor expansion. The principal effect of the antisense oligonucleotides was a reduction of overall colony numbers. The size of the individual colonies was also reduced.

Antisense oligonucleotide experiments using both human and murine stem cells demonstrated an inhibition of myeloid colony formation. Although, the reduction in myelopoiesis observed in these assays could be prevented by the additional inclusion of G-CSF and GM-CSF in the culture medium. These data serve to illustrate the redundancy of cytokine action in the myelopoietic compartment.

TABLE 1

EXPERIMENT	OLIGO	AVG. COLONY #	% INHIBITION
Human Cord Blood (KL)	(-20)AS	32	
	(-20)S	100	70
	(-20)SCR	114	
	(+85)AS	80	
	(+85)S	123	38
	(+85)SCR	138	
	Control	158	
Human Cord Blood (IL-3, IL-6, KL)	(-20)AS	78	
	(-20)S	188	54
	(-20)SCR	151	
	(+85)AS	167	
	(+85)S	195	18
	(+85)SCR	213	
	Control	266	
Human Cord Blood (KL)	(-20)AS	42	
	(-20)S	146	69
	(-20)SCR	121	
	(+85)AS	123	
	(+85)S	162	23
	(+85)SCR	156	
	Control	145	
Murine Fetal Liver (KL)	(+84)AS	33	
	(+84)S	86	54
	(+84)SCR	57	
	(-20)AS	27	
	(-20)S	126	71
	(-20)SCR	60	
	(-99)AS	109	
	(-99)S	93	0
	(-99)SCR	109	
Murine Fetal Liver (KL)	Control	121	
	(-213)AS	51	
	(-213)S	60	10
	(-213)SCR	53	
	(+211)AS	58	
	(+211)S	54	3
	(+211)SCR	66	
	Control	59	

#### Materials and Methods

10 *Human stem cells:* Human umbilical cord blood was collected in PBS/Heparin (1000 $\mu$ /ml). The mononuclear fraction was separated using a dextran gradient and any remaining red blood cells lysed in 20 mM NH<sub>4</sub> Cl. CD34<sup>+</sup> cells were isolated using CD34<sup>+</sup> immunomagnetic beads (Miltenyi, CA). These isolated CD34<sup>+</sup> cells were found to be 90-97% CD34<sup>+</sup> by FACS analysis.

*Murine stem cells:* Midgestation fetal liver were harvested and positively selected for the AA4<sup>+</sup> antigen by immune panning. The AA4<sup>+</sup> positive fraction was then further enriched for stem cell content by FACS isolation of the AA4<sup>+</sup> Sca<sup>+</sup> Kit<sup>+</sup> fraction.

*Antisense experiments:* Oligodeoxynucleotides were synthesized against regions of the human or murine WSX receptors. For each oligonucleotide chosen, antisense (AS), sense (S) and scrambled (SCR) versions were synthesized (see Fig. 7). + or - indicates position relative the initiation methionine of the WSX receptor. CD34<sup>+</sup> or AA4<sup>+</sup> Sca<sup>+</sup> Kit<sup>+</sup> cells were incubated at a concentration of 10<sup>3</sup>/ml in 50:50 DMEM/F12 media supplemented with 10% FBS, L-glutamine, and GIBCO™ lipid concentrate containing either sense, antisense or scrambled oligonucleotides at a concentration of 70 µg/ml. After 16 hours, a second aliquot of the respective oligonucleotide was added (35 µg/ml) and the cells incubated for a further 6 hours.

*Colony assays:* 5000 cells from each of the above conditions were aliquoted into 5 ml of methylcellulose (Stem Cell Technologies) containing kit ligand (KL) (25 ng/ml), interleukin-3 (IL-3) (25 ng/ml) and interleukin-6 (IL-6) (50 ng/ml). The methylcellulose cultures were then incubated at 37°C for 14 days and the resultant colonies counted and phenotyped. All assays were performed in triplicate.

#### EXAMPLE 9

##### WSX Receptor Variant 13.2 is a Receptor for OB Protein

The WSX receptor variant 13.2 has essentially the same amino acid sequence as the recently cloned leptin (OB) receptor. See Tartaglia *et al.*, *Cell* 83:1263-1271 (1995). OB protein was able to stimulate thymidine incorporation in Baf3 cells transfected with WSX receptor variant 13.2 as described in Example 4 (See Fig. 9).

OB protein expression in hematopoietic cells was studied. Oligonucleotide primers designed specifically against the OB protein illustrated the presence of this ligand in fetal liver and fetal brain as well as in two fetal liver stromal cell lines, designated 10-6 and 7-4. Both of these immortalized stromal cell lines have been demonstrated to support both myeloid and lymphoid proliferation of stem cell populations (Zeigler *et al.*, *Blood* 84:2422-2430 (1994)).

#### EXAMPLE 10

##### Role of OB Protein in Hematopoiesis

To examine the hematopoietic activity of OB protein, a variety of *in vitro* assays were performed.

Murine fetal liver fLASK stem cells were isolated from the midgestational fetal liver as described in Zeigler *et al.*, *Blood* 84:2422-2430 (1994) and studied in stem cell suspension culture or methylcellulose assays.

For the stem cell suspension cultures, twenty thousand of the fLASK cells were seeded in individual wells in a 12 well format in DMEM 4.5/F12 media supplemented with 10% heat inactivated fetal calf serum (Hyclone, Logan, UT) and L-glutamine. Growth factors were added at the following concentrations: kit ligand (KL) at 25 ng/mL, interleukin-3 (IL-3) at 25 ng/mL, interleukin-6 (IL-6) at 50 ng/mL, G-CSF at 100 ng/mL, GM-CSF at 100 ng/mL, EPO at 2U/mL, interleukin-7 (IL-7) at 100 ng/mL (all growth factors from R and D Systems,

Minneapolis, MN). OB protein was added at 100 ng/mL unless indicated otherwise. Recombinant OB protein was produced as described in Levin *et al.*, *Proc. Natl. Acad. Sci. (USA)* 93:1726-1730 (1996).

In keeping with its ability to transduce a proliferative signal in Baf3 cells (see previous Example), OB protein dramatically stimulated the expansion of fASK cells grown in suspension culture in the presence of kit ligand (Fig. 10A). The addition of OB protein alone to these suspension cultures was unable to effect survival of the hematopoietic stem cells (HSCs). When a variety of hematopoietic growth factors in suspension culture assays were tested, the main synergy of OB protein appeared to be with KL, GM-CSF and IL-3 (Table 2). No preferential expansion of any particular lineage was observed from cytopsin analysis of the resultant cultures.

TABLE 2

Factor	KL	KL+OB protein	OB protein
N/A	128+/-9	192+/-13	
G-CSF	131+/-3	177+/-8	30+/-5
GM-CSF	148+/-4	165+/-6	134+/-10
IL-3	189+/-7	187+/-4	144+/-
IL-6	112+/-4	198+/-5	32+/-3
EPO	121+/-3	177+/-8	30+/-6
IL-3 & IL-6	112+/-12	198+/-7	32+/-7

fASK stem cells were isolated. Twenty thousand cells were plated in suspension culture with the relevant growth factor combination. Cells were harvested and counted after 7 days. Cell numbers are presented  $\times 10^3$ . Assays were performed in triplicate and repeated in two independent experiments.

Methylcellulose assays were performed as previously described (Zeiger *et al.*, *supra*). Briefly, methylcellulose colony assays were performed using "complete" methylcellulose or pre-B methylcellulose medium (Stem Cell Technologies, Vancouver, British Columbia, Canada) with the addition of 25 ng/mL KL (R and D Systems, Minneapolis, MN). Cytospin analyses of the resultant colonies were performed as previously described in Zeigler *et al.*

When these methylcellulose assays were employed, OB protein augmented myeloid colony formation and dramatically increased lymphoid and erythroid colony formation (Figs. 10B and 10C) which demonstrates that OB protein can act on very early cells of the hematopoietic lineage. Importantly, the hematopoietic activity of OB protein was not confined to fetal liver stem cells, the murine bone marrow stem cell population; Lin<sup>lo</sup>Sca<sup>+</sup> also proliferated in response to OB protein (KL: 5 fold expansion, KL and OB protein: 10 fold expansion).

Further hematopoietic analysis of the role of the WSX receptor was carried out by examining hematopoietic defects in the *db/db* mouse.

These defects were assessed by measuring the proliferative potential of *db/db* homozygous mutant marrow. Under conditions favoring either myeloid (Humphries *et al.*, *Proc. Natl. Acad. Sci. (USA)* 78:3629-3633 (1981)) or lymphoid (McNiece *et al.*, *J. Immunol.* 146:3785-90 (1991)) expansion, the colony forming potential of the *db/db* marrow was significantly reduced when compared to the wild-type control marrow (Fig. 11). This was particularly evident when the comparison was made under pre-B methylcellulose conditions where KL and IL-7 are used to drive lymphopoiesis (McNiece *et al.*, *supra*). Corresponding analysis of the complementary mouse mutation *ob/ob*, which is deficient in the production of OB protein (Zhang *et al.*, *Nature* 372:425-431 (1994)), also indicated that the lymphoproliferative capacity is compromised in the absence of a functional OB protein signalling pathway (Fig. 11). However, this reduction was less than the reduction observed using *db/db* marrow.

Analysis of the cellular profile of the *db/db* and wild-type marrow revealed significant differences between the two. Overall cellularity of the *db/db* marrow was unchanged. However, when various B cell populations in the *db/db* marrow were examined, both decreased levels of B220<sup>+</sup> and B220<sup>+</sup>/CD43<sup>+</sup> cells were found. B220<sup>+</sup> cells represent all B cell lineages while CD43 is considered to be expressed preferentially on the earliest cells of the B cell hierarchy (Hardy *et al.*, *J. Exp. Med.* 173:1213-25 (1991)). No differences were observed between the CD4/CD8 staining profiles of the two groups. The TER119 (a red cell lineage marker) population was increased in the *db/db* marrow (Fig. 12A).

Comparison of the spleens from the two groups revealed a significant decrease in both tissue weight and cellularity of the *db/db* mice compared to the homozygote misty gray controls (0.063 ± 0.009 g vs. 0.037 ± 0.006 g and  $1.10 \times 10^7 \pm 1 \times 10^4$  vs.  $4.3 \times 10^6 \pm 10^3$  cells > p0.05). This decreased cellularity in the *db* spleen was reflected in a marked reduction in TER119 staining (Fig. 12B). This result appears to confirm the synergy demonstrated between OB protein and EPO and points to a role for OB protein in the regulation of erythropoiesis.

Examination of the hematopoietic compartment of the *db/db* mouse *in vivo* demonstrated a significant reduction in peripheral blood lymphocytes when compared to heterozygote or wild-type controls. *Db/db* mice fail to regulate blood glucose levels and become diabetic at approximately 6-8 weeks of age; therefore, peripheral blood counts as the animals matured were followed.

For procurement of blood samples, prior to the experiment and at time points throughout the study, 40 µL of blood was taken from the orbital sinus and immediately diluted into 10 mL of diluent to prevent clotting. The complete blood count from each blood sample was measured on a Serrono Baker system 9018 blood analyzer within 60 min. of collection. Only half the animals in each dose group were bled on any given day, thus, each animal was bled on alternate time points. Blood glucose levels were measured in orbital sinus blood samples using One Touch glucose meters and test strips (Johnson and Johnson). The results of this experiment are shown in Figs. 13A-C.

This analysis demonstrated that peripheral blood lymphocytes are significantly reduced at all time points compared to control animals and that the peripheral lymphocyte population of the *db/db* mouse does not change significantly with age. FACS analysis revealed that the decreased lymphocyte population represented a decrease in both B220<sup>+</sup> cells and CD4/CD8 cells. Both erythrocyte and platelets are at wild-type levels throughout all time periods examined. The peripheral blood lymphocyte levels in *ob/ob* homozygous mutant mice were unchanged from wild-type controls.

Hematopoietic analysis of the *db/db* mouse can be complicated by the onset of diabetes. Therefore, the impact of high glucose levels on lymphopoiesis was examined by comparing the peripheral blood profiles and blood glucose levels in two other diabetic models, the glucokinase knockout heterozygote mouse (Grupe *et al.*, *Cell* 83:69-78 (1995)) and the IFN- $\alpha$  transgenic mouse (Stewart *et al.*, *Science* 260:1942-6 (1993)). Comparison of peripheral lymphocytes and blood glucose in *db/db* mice, their appropriate controls and the high glucose models illustrated no relationship between blood-glucose and lymphocyte counts (Fig. 14). These results suggest therefore that the lymphoid defects observed in the *db/db* mouse are directly attributed to the hematopoietic function of the OB protein signalling pathway.

To test the capacity of the *db/db* hematopoietic compartment to respond to challenge, the *db/db* mice and controls were subjected to sub-lethal irradiation C57BLKS/J *db/db*, C57BLKS/Jm<sup>+</sup>/db, and C57BLKS/J<sup>+</sup>m<sup>+</sup>/m mice were subjected to sub-lethal whole body irradiation (750 cGy, 190 cGy/min) as a single dose from a <sup>137</sup>Cs source. Ten animals were used per experimental group. The kinetics of hematopoietic recovery were then followed by monitoring the peripheral blood during the recovery phase. This experiment illustrated the inability of the *db/db* hematopoietic system to fully recover the lymphopoietic compartment of the peripheral blood 35 days post-irradiation. Platelet levels in these mice followed the same recovery kinetics as controls, however the reduction in erythrocytes lagged behind controls by 7-10 days. This finding may reflect the increased TER 119 population found in the marrow of the *db/db* mice (Fig. 12A).

#### Materials and Methods

Bone marrow, spleens and peripheral blood was harvested from the diabetic mouse strains: C57BLKS/J *db/db* (mutant), C57BLKS/J m<sup>+</sup>/db (lean heterozygote control littermate), C57BLKS/J+m<sup>+</sup>/m (lean homozygote misty gray coat control littermate) and the obese mouse strains: C57BL/6J-*ob/ob* (mutant) and the C57BL/6J-*ob/+* (lean littermate control). All strains from the Jackson Laboratory, Bar Harbor, ME. A minimum of five animals were used per experimental group. Femurs were flushed with Hank's balanced salt solution (HBSS) plus 2% FCS and a single cell suspension was made of the bone marrow cells. Spleens were harvested and the splenic capsule was ruptured and filtered through a nylon mesh. Peripheral blood was collected through the retro-orbital sinus in phosphate buffered saline (PBS) with 10U/mL heparin and Immol EDTA and processed as previously described. The bone marrow, splenocytes and peripheral blood were then stained with the monoclonal antibodies against the following antigens: B220/CD45R (Pan B cell) FITC antimouse, TER-119/erythroid cell R-PE antimouse, CD4 (L3T4), FITC antimouse, CD8 (Ly 3.2), FITC antimouse, and sIgM (Igh-6b), FITC antimouse



(All monoclonals from Pharmigen, San Diego, CA). The appropriate isotype controls were included in each experiment. For methylcellulose assays, the bone marrow from five animals per group was pooled and 100,000 cell aliquots from each group used for each assay point.

#### EXAMPLE 11

##### Expression of OB-immunoadhesin

Using protein engineering techniques, the human OB protein was expressed as a fusion with the hinge, CH2 and CH3 domains of IgG1. DNA constructs encoding the chimera of the human OB protein and IgG1 Fc domains were made with the Fc region clones of human IgG1. Human OB cDNA was obtained by PCR from human fat cell cDNA (Clontech Buick-Clone cDNA product). The source of the IgG1 cDNA was the plasmid pBSSK-CH2CH3. The chimera contained the coding sequence of the full length OB protein (amino acids 1-167 in Figure 16) and human IgG1 sequences beginning at aspartic acid 216 (taking amino acid 114 as the first residue of the heavy chain constant region (Kabat *et al.*, *Sequences of Proteins of Immunological Interest* 4th ed. (1987)), which is the first residue of the IgG1 hinge after the cysteine residue involved in heavy-light chain bonding, and ending with residues 441 to include the CH2 and CH3 Fc domains of IgG1. There was an insert of codons for three amino acids (GlyValThr) between the OB protein and IgG1 coding sequences. If necessary, this short linker sequence can easily be deleted, for example by site directed deletion mutagenesis, to create an exact junction between the coding sequences of the OB protein and the IgG1 hinge region. The coding sequence of the OB-IgG1 immunoadhesin was subcloned into the pRK5-based vector pRK5tk-neo which contains a neomycine selectable marker, for transient expression in 293 cells using the calcium phosphate technique (Suva *et al.*, *Science* 237:893-896 (1987)). 293 cells were cultured in HAM's : Low Glucose DMEM medium (50:50), containing 10% FBS and 2 mM L-Gln. For purification of OB-IgG1 chimeras, cells were changed to serum free production medium PS24 the day after transfection and media collected after three days. The culture media was filtered.

The filtered 293 cell supernatant (400 ml) containing recombinant human OB-IgG1 was made 1 mM in phenylmethylsulfonyl fluoride and 2 µg/ml in aprotinin. This material was loaded at 4°C onto a 1 x 4.5 cm Protein A agarose column (Pierce catalog # 20365) equilibrated in 100 mM HEPES pH 8. The flow rate was 75 ml/h. Once the sample was loaded, the column was washed with equilibration buffer until the A<sub>280</sub> reached baseline. The OB-IgG1 protein was eluted with 3.5 M MgCl<sub>2</sub> + 2% glycerol (unbuffered) at a flow rate of 15 ml/h. The eluate was collected with occasional mixing into 10 ml of 100 mM HEPES pH 8 to reduce the MgCl<sub>2</sub> concentration by approximately one-half and to raise the pH. The eluted protein was then dialyzed into phosphate buffered saline, concentrated, sterile filtered and stored either at 4°C or frozen at -70 °C. The OB-IgG1 immunoadhesin prepared by this method is estimated by SDS-PAGE to be greater than 90% pure.

**EXAMPLE 12****Preparation of PEG-OB**

The PEG derivatives of the human OB protein were prepared by reaction of hOB protein purified by reverse phase chromatography with a succinimidyl derivative of PEG propionic acid (SPA-PEG) having a nominal molecular weight of 10 kD, which had been obtained from Shearwater Polymers, Inc. (Huntsville, AL). After purification of the hOB protein by reverse phase chromatography, an approximately 1-2 mg/ml solution of the protein in 0.1% trifluoroacetic acid and approximately 40% acetonitrile, was diluted with 1/3 to 1/2 volume of 0.2 M borate buffer and the pH adjusted to 8.5 with NaOH. SPA-PEG was added to the reaction mixture to make 1:1 and 1:2 molar ratios of protein to SPA-PEG and the mixture was allowed to incubate at room temperature for one hour. After reaction and purification by gel electrophoresis or ion exchange chromatography, the samples were extensively dialyzed against phosphate-buffered saline and sterilized by filtration through a 0.22 micron filter. Samples were stored at 4°C. Under these conditions, the PEG-hOB resulting from the 1:1 molar ratio protein to SPA-PEG reaction consisted primarily of molecules with one 10 kD PEG attached with minor amounts of the 2 PEG-containing species. The PEG-hOB from the 1:2 molar reaction consisted of approximately equal amounts of 2 and 3 PEGs attached to hOB, as determined by SDS gel electrophoresis. In both reactions, small amounts of unreacted protein were also detected. This unreacted protein can be efficiently removed by the gel filtration or ion exchange steps as needed. The PEG derivatives of the human OB protein can also be prepared essentially following the aldehyde chemistry described in EP 372,752 published June 13, 1990.

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**EXAMPLE 13****Murine Agonist Antibodies**

Mice were immunized five times with 20µg of the WSX receptor immunoadhesin (see Example 2 above) resuspended in MPL-TDM (monophosphoryl lipid A/trehalose dicorynomycolate; Rabi, Immunochemical Research Inc.) into each foot pad. Three days after the last immunization, popliteal lymphoid cells were fused with mouse myeloma cells, X63-Ag8.8.653 cells, using 50% polyethylene glycol as described (Laskov *et al. Cell. Immunol.* 55:251 (1980)).

The initial screening of hybridoma culture supernatants was done using a capture ELISA. For the capture ELISA, microtiter plates (Maxisorb; Nunc, Kamstrup, Denmark) were coated with 50µl/well of 2µg/ml of goat antibodies specific to the Fc portion of human IgG (Goat anti-hIgG-Fc; Cappel), in PBS, overnight at 4°C and blocked with 2x BSA for 1 hr at room temperature. Then, 50µl/well of 2µg/ml of WSX receptor immunoadhesin was added to each well for 1 hr. The remaining anti-Fc binding sites were blocked with PBS containing 3% human serum and 10µg/ml of CD4-IgG for 1 hr. Plates were incubated with 50µl/well of 2µg/ml of anti-WSX receptor monoclonal antibody (or hybridoma culture supernatant) for 1 hr. Plates were then incubated with 50µl/well of HRP-goat anti-mouse IgG. The bound enzyme was detected by the addition of the

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substrate (OPD) and the plates were read at 490nm with an ELISA plate reader. Between each step, plates were washed in wash buffer (PBS containing 0.05% TWEEN 20™).

Agonist antibodies were screened for using the KIRA ELISA described in WO95/14930. A chimeric receptor comprising the extracellular domain of the WSX receptor and the transmembrane and intracellular domain of Rse receptor (Mark *et al.*, *Journal of Biological Chemistry* 269(14):10720-10728 (1994)) with a carboxyl-terminal herpes simplex virus glycoprotein D (gD) tag was produced and dp12.CHO cells were transformed therewith as described in Example 4 of WO95/14930.

The WSX/Rse.gD transformed dp12.CHO cells were seeded ( $3 \times 10^4$  per well) in the wells of a flat-bottom-96 well culture plate in 100µl media and cultured overnight at 37°C in 5% CO<sub>2</sub>. The following morning the well supernatants were removed and various concentrations of purified mAb were then added to separate wells. The cells were stimulated at 37°C for 30 min. and the well supernatants were decanted. To lyse the cells and solubilize the chimeric receptors, 100 µl of lysis buffer was added to each well. The plate was then agitated gently on a plate shaker (Bellco Instruments, Vineland, NJ) for 60 min. at room temperature.

While the cells were being solubilized, an ELISA microtiter plate (Nunc Maxisorp, Inter Med, Denmark) coated overnight at 4°C with the 5B6 monoclonal anti-gD antibody (5.0 µg/ml in 50 mM carbonate buffer, pH 9.6, 100 µl/well) was decanted and blocked with 150 µl/well of Block Buffer containing 2% BSA for 60 min. at room temperature. After 60 minutes, the anti-gD 5B6 coated plate was washed 6 times with wash buffer (PBS containing 0.05 % TWEEN 20™ and 0.01 % thimerosal).

The lysate containing solubilized WSX/Rse.gD from the cell-culture microtiter well was transferred (85µl/well) to anti-gD 5B6 coated and blocked ELISA well and was incubated for 2 h at room temperature. The unbound WSX/Rse.gD was removed by washing with wash buffer and 100 µl of biotinylated 4G10 (anti-phosphotyrosine) diluted 1:18000 in dilution buffer (PBS containing 0.5 % BSA, 0.05 % Tween-20, 5 mM EDTA, and 0.01 % thimerosal), *i.e.* 56 ng/ml was added to each well. After incubation for 2 h at room temperature the plate was washed and HRPO-conjugated streptavidin (Zymed Laboratories, S. San Francisco, CA) was added to each well. The plate was incubated for 30 minutes at room temperature with gentle agitation. The free avidin-conjugate was washed away and 100 µl freshly prepared substrate solution (tetramethyl benzidine (TMB); 2-component substrate kit; Kirkegaard and Perry, Gaithersburg, MD) was added to each well. The reaction was allowed to proceed for 10 minutes, after which the color development was stopped by the addition of 100µl/well 1.0 M H<sub>3</sub>PO<sub>4</sub>. The absorbance at 450 nm was read with a reference wavelength of 650 nm (ABS<sub>450/650</sub>), using a *vmax* plate reader (Molecular Devices, Palo Alto, CA) controlled with a Macintosh Centris 650 (Apple Computers, Cupertino, CA) and DeltaSoft software (BioMetallics, Inc, Princeton, NJ).

Four of the 25 anti-WSX receptor monoclonal antibodies activated the chimeric WSX/Rse receptor in the KIRA ELISA. The antibodies were designated: 2D7, 1G4, 1E11 and 1C11.

To determine whether the four agonist anti-WSX receptor mAbs recognized the same or different epitopes, a competitive binding ELISA was performed as described in Kim *et al. J. Immunol. Method* 156:9-17

(1992) using biotinylated mAbs (Bio-mAb). Bio-mAb were prepared using N-hydroxyl succinimide as described in *Antibodies, A Laboratory Manual* Cold Spring Harbor Laboratory, Eds. Harlow E. and D. Lane, p. 341 (1988). Microtiter wells were coated with 50µl of Goat anti-hIgG-Fc and kept overnight at 4°C, blocked with 2% BSA for 1 hr, and incubated with 25 µl/well of human WSX receptor immunoadhesin (1µg/ml) for 1 hr at room temperature. After washing, a mixture of a predetermined optimal concentration of Bio-mAb bound and a thousand-fold excess of unlabeled mAb was added into each well. Following 1hr incubation at room temperature, plates were washed and the amount of Bio-mAb was detected by the addition of HRP-streptavidin. After washing the plates, the bound enzyme was detected by the addition of the substrate o-phenylenediamine dihydrochloride (OPD), and the plates were read at 490nm with an ELISA plate reader.

The ability of the mAbs to recognize murine WSX receptor was determined in a capture ELISA. Murine WSX receptor (Fig. 21) fused to a gD tag (see above) was captured by an anti-gD (5B6) coated ELISA plate. After washing, various concentrations of biotinylated mAbs were added into each well. Biotinylated mAbs bound to murine WSX receptor-gD were detected using HRP-streptavidin as described above.

To determine whether the antibodies bound membrane-bound receptor, FACS analysis was performed using 293 cells transfected with WSX receptor. 10<sup>5</sup> WSX receptor-transfected 293 cells were resuspended in 100µl of PBS plus 1% fetal calf serum (FCS) and incubated with 2D7 or 1G4 hybridoma cell supernatant for 30 min on ice. After washing, cells were incubated with 100µl of FITC-goat anti-mouse IgG for 30 min at 4°C. Cells were washed twice and resuspended in 150µl of PBS plus 1% FCS and analyzed by FACscan (Becton Dickinson, Mountain View, CA). The antibodies 2D7 and 1G4 bound to membrane WSX receptor according to the FACS analysis.

The properties of agonist antibodies 2D7 and 1G4 are summarized in the following table.

TABLE 2

mAb	Isotype	epitope <sup>a</sup>	hWSXR <sup>b</sup>	mWSXR <sup>b</sup>	Agonist <sup>c</sup>
2D7	IgG1	A	+++	++	+
1G4	IgG1	B	+++	+	+

<sup>a</sup> These mAbs are shown to recognize different epitopes by competitive binding ELISA.

<sup>b</sup> These results are determined by ELISA (hWSXR is human WSX receptor and mWSXR is murine WSX receptor).

<sup>c</sup> The agonistic activities were determined by KIRA ELISA.

#### EXAMPLE 14

##### Human Agonist Antibodies

Single-chain Fv (scFv) fragments binding to the human WSX receptor (hWSXR) were isolated from a large human scFv library (Vaughan *et al.* *Nature Biotechnology* 14:309-314 (1996)) using antigen coated on immunotubes or biotinylated antigen in conjunction with streptavidin-coated magnetic beads (Griffiths *et al.*

*EMBO J.* 13:3245–3260 (1994); and Vaughan *et al.* (1996)). Briefly, immunotubes coated overnight with 10 µg/ml human WSX receptor immunoadhesin (see Example 2 above) in phosphate buffered saline (PBS) were used for three rounds of panning. The humanized antibody, huMAb4D5–8 (Carter *et al. Proc. Natl. Acad. Sci. USA* 89:4285–4289 (1992)) was used to counter-select for antibodies binding to the Fc of the immunoadhesin.

- 5 This was done by using 1mg/ml huMAb4D5-8 in solution for the panning steps. In addition, human WSX receptor extracellular domain (cleaved from the WSX receptor immunoadhesin with Genenase (Carter *et al. Proteins: Structure, Function and Genetics* 6:240-248 (1989)) was biotinylated and used for three rounds of panning. Individual phage following two or three rounds of panning were characterized by antigen-binding ELISA (Tables 3 and 4).

10

TABLE 3

## Panning with human WSX receptor immunoadhesin-coated immunotubes

Phage ELISA			# clones characterized	# BstNI fingerprints
Round	hWSXR	Fc		
2	74 / 96	0 / 96	74	11 <sup>a</sup>
15 3	191 / 192	1 / 192	58	8 <sup>a</sup>

<sup>a</sup> Total of 11 different clones identified.

TABLE 4

## Panning with biotinylated human WSX receptor

Phage ELISA			# clones characterized	# BstNI fingerprints
Round	hWSXR	Fc		
2	8 / 96	0 / 96	8	4 <sup>a</sup>
20 3	49 / 192	1 / 192	49	4 <sup>a</sup>

<sup>a</sup> Total of 7 different clones identified.

- Clones binding to human WSX receptor were further characterized by BstNI fingerprinting of a PCR  
 25 fragment encoding the scFv. A total of 18 clones were identified: 11 from the panning using immunotubes and 7 from the panning using biotinylated antigen (there was no overlap between these groups). The DNA for all 18 clones was sequenced.

- Anti-huWSXR clones obtained as described above were analyzed for agonist activity in a KIRA-ELISA  
 assay (see above and Fig. 22) firstly as scFv phage and then as scFv. The scFv phage were PEG-precipitated  
 30 (Carter *et al., Mutagenesis: A Practical Approach*, McPherson, M. ed. IRL Press, Oxford, UK, Chapter 1, pp 1-25 (1991)) and resuspended in PBS prior to screening. To prepare the scFv, DNA from the clones was transformed into 33D3 cells (a non-suppressor strain for expression of soluble protein). The cells were plated

onto 2YT/2%glucose/50µg per ml of carbenicillin and incubated at 37°C overnight. A 5 ml culture (2YTG: 2YT, 2% glucose, 50µg/ml carbenicillin) was inoculated and grown at 30°C overnight. The next morning, the 5ml culture was diluted into 500ml 2YTG media and grown at 30°C until OD550 = 0.3. Then, the media was changed from 2YTG into 2YT/50µg/ml carbenicillin/2mM IPTG and grown at 30°C for 4-5 hrs for scFv production. The culture was harvested and the cell pellet was frozen at -20°C. For purification, the cell pellet was resuspended in 10ml shockate buffer (50mM TrisHCl pH8.5, 20% sucrose, 1mM EDTA) and agitated at 4°C for 1hr. The debris was spun down and supernatant was taken to be purified on Ni NTA Superose (Qiagen) column. MgCl<sub>2</sub> was added to the supernatant to 5mM and loaded onto 0.5ml Ni NTA Superose packed into a disposable column. The column was then washed with 2x5ml wash buffer 1 (50mM sodium phosphate, 300mM NaCl, 25mM imidazole pH 8.0) followed by 2x5ml wash 2 buffer (50mM sodium phosphate, 300mM NaCl, 50mM imidazole pH 8.0). The scFv was then eluted with 2.5ml elution buffer (50mM sodium phosphate, 300mM NaCl, 250mM imidazole, pH8.0). The eluted pool was buffer exchanged into PBS with a NAP5 column (Pharmacia) and stored at 4°C.

Clones #3, #4 and #17 were found to have agonist activity as phage and as scFv (see Figs. 23 and 24). The sequences of these agonist clones are shown in Fig. 25. The activity of the antibodies as F(ab')<sub>2</sub> in the KIRA ELISA was assessed, with clone #4 and clone # 17 showing enhanced activity as F(ab')<sub>2</sub>. The ability of the antibodies to bind murine WSX receptor in a capture ELISA (see Example 13) was assessed. Clone #4 and clone # 17 bound murine WSX receptor in this assay.

## SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT: GENENTECH, INC.
  - (ii) TITLE OF INVENTION: WSX RECEPTOR AND LIGANDS
  - 5 (iii) NUMBER OF SEQUENCES: 51
  - (iv) CORRESPONDENCE ADDRESS:
    - (A) ADDRESSEE: Genentech, Inc.
    - (B) STREET: 460 Point San Bruno Blvd
    - (C) CITY: South San Francisco
    - 10 (D) STATE: California
    - (E) COUNTRY: USA
    - (F) ZIP: 94080
  - (v) COMPUTER READABLE FORM:
    - (A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
    - 15 (B) COMPUTER: IBM PC compatible
    - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
    - (D) SOFTWARE: WinPatin (Genentech)
  - (vi) CURRENT APPLICATION DATA:
    - (A) APPLICATION NUMBER:
    - 20 (B) FILING DATE:
    - (C) CLASSIFICATION:
  - (vii) PRIOR APPLICATION DATA:
    - (A) APPLICATION NUMBER: 08/667197
    - (B) FILING DATE: 06/20/96
  - 25 (vii) PRIOR APPLICATION DATA:
    - (A) APPLICATION NUMBER: 08/585005
    - (B) FILING DATE: 01/08/96
  - (viii) ATTORNEY/AGENT INFORMATION:
    - (A) NAME: Lee, Wendy M.
    - 30 (B) REGISTRATION NUMBER: 40,378
    - (C) REFERENCE/DOCKET NUMBER: P0986P2PCT
  - (ix) TELECOMMUNICATION INFORMATION:
    - (A) TELEPHONE: 415/225-1994
    - (B) TELEFAX: 415/952-9881
    - 35 (C) TELEX: 910/371-7168
- (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 4102 base pairs
    - (B) TYPE: Nucleic Acid
    - 40 (C) STRANDEDNESS: Double
    - (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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 AGTGGGGCTA TTGGACTGAC TTTTCTTATG CTGGGATGTG CCTTAGAGGA 100  
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 CAACCTATGA CTACTTCCTT TTGCCTGCTG GACTCTCAA GAATACTTCA 300  
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 TGGTACTCAC TTTTCTAACT TATCCAAAAC AACTTTCCAC TGTGCTTTC 400  
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 GTCCATCTTT TATATGTTCT GCCTGAAGTG TTAGAAGATT CACCTCTGGT 650  
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 CTCCTTATGT GTTTGAAAAT CACATCTGGT GGAGTAATTT TCCAGTCACC 800  
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 20 AGCCCAACAT TGGTACCATT TCCACTTCAA TATCAAGTGA AATATTCAGA 950  
 GAATTCTACA ACAGTTATCA GAGAAGCTGA CAAGATTGTC TCAGCTACAT 1000  
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 25 CAAGTGTGG GTCTAATGTT TCTTTTCACT GCATCTATAA GAAGGAAAAC 1200  
 AAGATTGTTT CCTCAAAGA GATTGTTTGG TGGATGAATT TAGCTGAGAA 1250



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GTCACCAAGT GCTTCTCTAG CAAAAATCTT CCGTTGAAGG ATTCTTTCTC 3200

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TTCATAAGCC TACCAATGTA GACACGCTCT TCTATTTTAT TCCCAAGCTC 3850

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TCCTTGTTGTG TTTTGAGAGT ATATTATGTA TTTATATTTT GTGCTATCAG 4050

5 ACTGTAGGAT TTGAAGTAGG ACTTTCCTAA ATGTTTAAGA TAAACAGAAT 4100

TC 4102

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 1165 amino acids  
(B) TYPE: Amino Acid  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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					20					25				30		
	Trp	Arg	Phe	Lys	Leu	Ser	Cys	Met	Pro	Pro	Asn	Ser	Thr	Tyr	Asp	
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20	Tyr	Phe	Leu	Leu	Pro	Ala	Gly	Leu	Ser	Lys	Asn	Thr	Ser	Asn	Ser	
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					80					85				90		
25	Phe	Arg	Ser	Glu	Gln	Asp	Arg	Asn	Cys	Ser	Leu	Cys	Ala	Asp	Asn	
					95					100				105		
	Ile	Glu	Gly	Lys	Thr	Phe	Val	Ser	Thr	Val	Asn	Ser	Leu	Val	Phe	
					110					115				120		
30	Gln	Gln	Ile	Asp	Ala	Asn	Trp	Asn	Ile	Gln	Cys	Trp	Leu	Lys	Gly	
					125					130				135		
	Asp	Leu	Lys	Leu	Phe	Ile	Cys	Tyr	Val	Glu	Ser	Leu	Phe	Lys	Asn	
					140					145				150		

- 82 -

	Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His Glu Cys His His	
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	Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser	
	425	430 435
5	Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser	
	440	445 450
	Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu Arg	
	455	460 465
10	Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His	
	470	475 480
	Pro Ile Ser Glu Pro Lys Asp Cys Tyr Leu Gln Ser Asp Gly Phe	
	485	490 495
	Tyr Glu Cys Ile Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr	
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15	Met Trp Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro	
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	Pro Thr Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro	
	530	535 540
20	Ser Ser Val Lys Ala Glu Ile Thr Ile Asn Ile Gly Leu Leu Lys	
	545	550 555
	Ile Ser Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe	
	560	565 570
	Gln Ile Arg Tyr Gly Leu Ser Gly Lys Glu Val Gln Trp Lys Met	
	575	580 585
25	Tyr Glu Val Tyr Asp Ala Lys Ser Lys Ser Val Ser Leu Pro Val	
	590	595 600
	Pro Asp Leu Cys Ala Val Tyr Ala Val Gln Val Arg Cys Lys Arg	
	605	610 615
30	Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser Asn Pro Ala Tyr	
	620	625 630
	Thr Val Val Met Asp Ile Lys Val Pro Met Arg Gly Pro Glu Phe	
	635	640 645
	Trp Arg Ile Ile Asn Gly Asp Thr Met Lys Lys Glu Lys Asn Val	
	650	655 660

	Thr	Leu	Leu	Trp	Lys	Pro	Leu	Met	Lys	Asn	Asp	Ser	Leu	Cys	Ser	665	670	675
	Val	Gln	Arg	Tyr	Val	Ile	Asn	His	His	Thr	Ser	Cys	Asn	Gly	Thr	680	685	690
5	Trp	Ser	Glu	Asp	Val	Gly	Asn	His	Thr	Lys	Phe	Thr	Phe	Leu	Trp	695	700	705
	Thr	Glu	Gln	Ala	His	Thr	Val	Thr	Val	Leu	Ala	Ile	Asn	Ser	Ile	710	715	720
10	Gly	Ala	Ser	Val	Ala	Asn	Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	Met	725	730	735
	Ser	Lys	Val	Asn	Ile	Val	Gln	Ser	Leu	Ser	Ala	Tyr	Pro	Leu	Asn	740	745	750
	Ser	Ser	Cys	Val	Ile	Val	Ser	Trp	Ile	Leu	Ser	Pro	Ser	Asp	Tyr	755	760	765
15	Lys	Leu	Met	Tyr	Phe	Ile	Ile	Glu	Trp	Lys	Asn	Leu	Asn	Glu	Asp	770	775	780
	Gly	Glu	Ile	Lys	Trp	Leu	Arg	Ile	Ser	Ser	Ser	Val	Lys	Lys	Tyr	785	790	795
20	Tyr	Ile	His	Asp	His	Phe	Ile	Pro	Ile	Glu	Lys	Tyr	Gln	Phe	Ser	800	805	810
	Leu	Tyr	Pro	Ile	Phe	Met	Glu	Gly	Val	Gly	Lys	Pro	Lys	Ile	Ile	815	820	825
	Asn	Ser	Phe	Thr	Gln	Asp	Asp	Ile	Glu	Lys	His	Gln	Ser	Asp	Ala	830	835	840
25	Gly	Leu	Tyr	Val	Ile	Val	Pro	Val	Ile	Ile	Ser	Ser	Ser	Ile	Leu	845	850	855
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5	Thr Asp Leu Glu Lys Gly Ser Val Cys Ile Ser Asp Gln Phe Asn	
	950	955 960
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10	Asp Glu Ser Gln Arg Gln Pro Phe Val Lys Tyr Ala Thr Leu Ile	
	980	985 990
	Ser Asn Ser Lys Pro Ser Glu Thr Gly Glu Glu Gln Gly Leu Ile	
	995	1000 1005
	Asn Ser Ser Val Thr Lys Cys Phe Ser Ser Lys Asn Ser Pro Leu	
	1010	1015 1020
15	Lys Asp Ser Phe Ser Asn Ser Ser Trp Glu Ile Glu Ala Gln Ala	
	1025	1030 1035
	Phe Phe Ile Leu Ser Asp Gln His Pro Asn Ile Ile Ser Pro His	
	1040	1045 1050
20	Leu Thr Phe Ser Glu Gly Leu Asp Glu Leu Leu Lys Leu Glu Gly	
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25	Asp Lys Ser Arg Val Ser Cys Pro Phe Pro Ala Pro Cys Leu Phe	
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	1115	1120 1125
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35	(2) INFORMATION FOR SEQ ID NO:3:	

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 896 amino acids

(B) TYPE: Amino Acid

(D) TOPOLOGY: Linear

## 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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20	Ile	Glu	Gly	Lys	Thr	Phe	Val	Ser	Thr	Val	Asn	Ser	Leu	Val	Phe	
					110					115					120	
	Gln	Gln	Ile	Asp	Ala	Asn	Trp	Asn	Ile	Gln	Cys	Trp	Leu	Lys	Gly	
					125					130					135	
	Asp	Leu	Lys	Leu	Phe	Ile	Cys	Tyr	Val	Glu	Ser	Leu	Phe	Lys	Asn	
25					140					145					150	
	Leu	Phe	Arg	Asn	Tyr	Asn	Tyr	Lys	Val	His	Leu	Leu	Tyr	Val	Leu	
					155					160					165	
	Pro	Glu	Val	Leu	Glu	Asp	Ser	Pro	Leu	Val	Pro	Gln	Lys	Gly	Ser	
					170					175					180	
30	Phe	Gln	Met	Val	His	Cys	Asn	Cys	Ser	Val	His	Glu	Cys	Cys	Glu	
					185					190					195	
	Cys	Leu	Val	Pro	Val	Pro	Thr	Ala	Lys	Leu	Asn	Asp	Thr	Leu	Leu	
					200					205					210	
	Met	Cys	Leu	Lys	Ile	Thr	Ser	Gly	Gly	Val	Ile	Phe	Gln	Ser	Pro	
35					215					220					225	



	Leu Met Ser Val Gln Pro Ile Asn Met Val Lys Pro Asp Pro Pro	
	230	240
	Leu Gly Leu His Met Glu Ile Thr Asp Asp Gly Asn Leu Lys Ile	
	245	255
5	Ser Trp Ser Ser Pro Pro Leu Val Pro Phe Pro Leu Gln Tyr Gln	
	260	270
	Val Lys Tyr Ser Glu Asn Ser Thr Thr Val Ile Arg Glu Ala Asp	
	275	285
10	Lys Ile Val Ser Ala Thr Ser Leu Leu Val Asp Ser Ile Leu Pro	
	290	300
	Gly Ser Ser Tyr Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly	
	305	315
	Pro Gly Ile Trp Ser Asp Trp Ser Thr Pro Arg Val Phe Thr Thr	
	320	330
15	Gln Asp Val Ile Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly	
	335	345
	Ser Asn Val Ser Phe His Cys Ile Tyr Lys Lys Glu Asn Lys Ile	
	350	360
20	Val Pro Ser Lys Glu Ile Val Trp Trp Met Asn Leu Ala Glu Lys	
	365	375
	Ile Pro Gln Ser Gln Tyr Asp Val Val Ser Asp His Val Ser Lys	
	380	390
	Val Thr Phe Phe Asn Leu Asn Glu Thr Lys Pro Arg Gly Lys Phe	
	395	405
25	Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His Glu Cys His His	
	410	420
	Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser	
	425	435
30	Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser	
	440	450
	Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu Arg	
	455	465
	Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His	
	470	480

	Pro	Ile	Ser	Glu	Pro	Lys	Asp	Cys	Tyr	Leu	Gln	Ser	Asp	Gly	Phe	485	490	495
	Tyr	Glu	Cys	Ile	Phe	Gln	Pro	Ile	Phe	Leu	Leu	Ser	Gly	Tyr	Thr	500	505	510
5	Met	Trp	Ile	Arg	Ile	Asn	His	Ser	Leu	Gly	Ser	Leu	Asp	Ser	Pro	515	520	525
	Pro	Thr	Cys	Val	Leu	Pro	Asp	Ser	Val	Val	Lys	Pro	Leu	Pro	Pro	530	535	540
10	Ser	Ser	Val	Lys	Ala	Glu	Ile	Thr	Ile	Asn	Ile	Gly	Leu	Leu	Lys	545	550	555
	Ile	Ser	Trp	Glu	Lys	Pro	Val	Phe	Pro	Glu	Asn	Asn	Leu	Gln	Phe	560	565	570
	Gln	Ile	Arg	Tyr	Gly	Leu	Ser	Gly	Lys	Glu	Val	Gln	Trp	Lys	Met	575	580	585
15	Tyr	Glu	Val	Tyr	Asp	Ala	Lys	Ser	Lys	Ser	Val	Ser	Leu	Pro	Val	590	595	600
	Pro	Asp	Leu	Cys	Ala	Val	Tyr	Ala	Val	Gln	Val	Arg	Cys	Lys	Arg	605	610	615
20	Leu	Asp	Gly	Leu	Gly	Tyr	Trp	Ser	Asn	Trp	Ser	Asn	Pro	Ala	Tyr	620	625	630
	Thr	Val	Val	Met	Asp	Ile	Lys	Val	Pro	Met	Arg	Gly	Pro	Glu	Phe	635	640	645
	Trp	Arg	Ile	Ile	Asn	Gly	Asp	Thr	Met	Lys	Lys	Glu	Lys	Asn	Val	650	655	660
25	Thr	Leu	Leu	Trp	Lys	Pro	Leu	Met	Lys	Asn	Asp	Ser	Leu	Cys	Ser	665	670	675
	Val	Gln	Arg	Tyr	Val	Ile	Asn	His	His	Thr	Ser	Cys	Asn	Gly	Thr	680	685	690
30	Trp	Ser	Glu	Asp	Val	Gly	Asn	His	Thr	Lys	Phe	Thr	Phe	Leu	Trp	695	700	705
	Thr	Glu	Gln	Ala	His	Thr	Val	Thr	Val	Leu	Ala	Ile	Asn	Ser	Ile	710	715	720
	Gly	Ala	Ser	Val	Ala	Asn	Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	Met	725	730	735

	Ser	Lys	Val	Asn	Ile	Val	Gln	Ser	Leu	Ser	Ala	Tyr	Pro	Leu	Asn	
					740					745					750	
	Ser	Ser	Cys	Val	Ile	Val	Ser	Trp	Ile	Leu	Ser	Pro	Ser	Asp	Tyr	
					755					760					765	
5	Lys	Leu	Met	Tyr	Phe	Ile	Ile	Glu	Trp	Lys	Asn	Leu	Asn	Glu	Asp	
					770					775					780	
	Gly	Glu	Ile	Lys	Trp	Leu	Arg	Ile	Ser	Ser	Ser	Val	Lys	Lys	Tyr	
					785					790					795	
	Tyr	Ile	His	Asp	His	Phe	Ile	Pro	Ile	Glu	Lys	Tyr	Gln	Phe	Ser	
10					800					805					810	
	Leu	Tyr	Pro	Ile	Phe	Met	Glu	Gly	Val	Gly	Lys	Pro	Lys	Ile	Ile	
					815					820					825	
	Asn	Ser	Phe	Thr	Gln	Asp	Asp	Ile	Glu	Lys	His	Gln	Ser	Asp	Ala	
					830					835					840	
15	Gly	Leu	Tyr	Val	Ile	Val	Pro	Val	Ile	Ile	Ser	Ser	Ser	Ile	Leu	
					845					850					855	
	Leu	Leu	Gly	Thr	Leu	Leu	Ile	Ser	His	Gln	Arg	Met	Lys	Lys	Leu	
					860					865					870	
	Phe	Trp	Glu	Asp	Val	Pro	Asn	Pro	Lys	Asn	Cys	Ser	Trp	Ala	Gln	
20					875					880					885	
	Gly	Leu	Asn	Phe	Gln	Lys	Arg	Thr	Asp	Ile	Leu					
					890					895	896					

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 923 amino acids  
 (B) TYPE: Amino Acid  
 (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

30	Met	Ile	Cys	Gln	Lys	Phe	Cys	Val	Val	Leu	Leu	His	Trp	Glu	Phe	
	1				5					10					15	
	Ile	Tyr	Val	Ile	Thr	Ala	Phe	Asn	Leu	Ser	Tyr	Pro	Ile	Thr	Pro	
					20					25					30	
	Trp	Arg	Phe	Lys	Leu	Ser	Cys	Met	Pro	Pro	Asn	Ser	Thr	Tyr	Asp	
					35					40					45	

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	Gly Ser Ser Tyr Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly	
	305	310 315
	Pro Gly Ile Trp Ser Asp Trp Ser Thr Pro Arg Val Phe Thr Thr	
	320	325 330
5	Gln Asp Val Ile Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly	
	335	340 345
	Ser Asn Val Ser Phe His Cys Ile Tyr Lys Lys Glu Asn Lys Ile	
	350	355 360
10	Val Pro Ser Lys Glu Ile Val Trp Trp Met Asn Leu Ala Glu Lys	
	365	370 375
	Ile Pro Gln Ser Gln Tyr Asp Val Val Ser Asp His Val Ser Lys	
	380	385 390
	Val Thr Phe Phe Asn Leu Asn Glu Thr Lys Pro Arg Gly Lys Phe	
	395	400 405
15	Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His Glu Cys His His	
	410	415 420
	Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser	
	425	430 435
20	Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser	
	440	445 450
	Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu Arg	
	455	460 465
	Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His	
	470	475 480
25	Pro Ile Ser Glu Pro Lys Asp Cys Tyr Leu Gln Ser Asp Gly Phe	
	485	490 495
	Tyr Glu Cys Ile Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr	
	500	505 510
30	Met Trp Ile Arg Ile Asn His Ser Leu Gly Ser Leu Asp Ser Pro	
	515	520 525
	Pro Thr Cys Val Leu Pro Asp Ser Val Val Lys Pro Leu Pro Pro	
	530	535 540
	Ser Ser Val Lys Ala Glu Ile Thr Ile Asn Ile Gly Leu Leu Lys	
	545	550 555

	Ile Ser Trp Glu Lys Pro Val Phe Pro Glu Asn Asn Leu Gln Phe	
	560	565 570
	Gln Ile Arg Tyr Gly Leu Ser Gly Lys Glu Val Gln Trp Lys Met	
	575	580 585
5	Tyr Glu Val Tyr Asp Ala Lys Ser Lys Ser Val Ser Leu Pro Val	
	590	595 600
	Pro Asp Leu Cys Ala Val Tyr Ala Val Gln Val Arg Cys Lys Arg	
	605	610 615
10	Leu Asp Gly Leu Gly Tyr Trp Ser Asn Trp Ser Asn Pro Ala Tyr	
	620	625 630
	Thr Val Val Met Asp Ile Lys Val Pro Met Arg Gly Pro Glu Phe	
	635	640 645
	Trp Arg Ile Ile Asn Gly Asp Thr Met Lys Lys Glu Lys Asn Val	
	650	655 660
15	Thr Leu Leu Trp Lys Pro Leu Met Lys Asn Asp Ser Leu Cys Ser	
	665	670 675
	Val Gln Arg Tyr Val Ile Asn His His Thr Ser Cys Asn Gly Thr	
	680	685 690
20	Trp Ser Glu Asp Val Gly Asn His Thr Lys Phe Thr Phe Leu Trp	
	695	700 705
	Thr Glu Gln Ala His Thr Val Thr Val Leu Ala Ile Asn Ser Ile	
	710	715 720
	Gly Ala Ser Val Ala Asn Phe Asn Leu Thr Phe Ser Trp Pro Met	
	725	730 735
25	Ser Lys Val Asn Ile Val Gln Ser Leu Ser Ala Tyr Pro Leu Asn	
	740	745 750
	Ser Ser Cys Val Ile Val Ser Trp Ile Leu Ser Pro Ser Asp Tyr	
	755	760 765
30	Lys Leu Met Tyr Phe Ile Ile Glu Trp Lys Asn Leu Asn Glu Asp	
	770	775 780
	Gly Glu Ile Lys Trp Leu Arg Ile Ser Ser Ser Val Lys Lys Tyr	
	785	790 795
	Tyr Ile His Asp His Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser	
	800	805 810

	Leu	Tyr	Pro	Ile	Phe	Met	Glu	Gly	Val	Gly	Lys	Pro	Lys	Ile	Ile	
							815				820				825	
	Asn	Ser	Phe	Thr	Gln	Asp	Asp	Ile	Glu	Lys	His	Gln	Ser	Asp	Ala	
							830				835				840	
5	Gly	Leu	Tyr	Val	Ile	Val	Pro	Val	Ile	Ile	Ser	Ser	Ser	Ile	Leu	
							845				850				855	
	Leu	Leu	Gly	Thr	Leu	Leu	Ile	Ser	His	Gln	Arg	Met	Lys	Lys	Leu	
							860				865				870	
	Phe	Trp	Glu	Asp	Val	Pro	Asn	Pro	Lys	Asn	Cys	Ser	Trp	Ala	Gln	
10							875				880				885	
	Gly	Leu	Asn	Phe	Gln	Lys	Met	Phe	Arg	Thr	Pro	Arg	Ile	Val	Pro	
							890				895				900	
	Gly	His	Lys	Asp	Leu	Ile	Phe	Arg	Arg	Cys	Leu	Lys	Ala	Ala	Cys	
							905				910				915	
15	Ser	Leu	Arg	Val	Ile	Thr	Thr	Pro								
							920				923					

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 3004 base pairs  
 (B) TYPE: Nucleic Acid  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATTCCGGG TTAAAGCTCT CGTGGCATTG TCCTTCAGTG GGGCTATTGG 50

25 ACTGACTTTT CTTATGCTGG GATGTGCCTT AGAGGATTAT GGATTGCCA 100

G TTCACCCTG ACCATCTTGA AAATAAGTTA TCTCTGATCT CTGTCTGTAT 150

GTTACTTCTC TCCCCTCACC AATGGAGAAC AAATGTGGGC AAAGTG TACT 200

TCTCTGAAGT AAGATGATTT GTCAAAAATT CTGTGTGGTT TTGTTACATT 250

GGGAATTTAT TTATGTGATA ACTGCGTTTA ACTTGT CATA TCCAATTACT 300

30 CCTTGGAGAT TTAAGTTGTC TTGCATGCCA CCAAATTCAA CCTATGACTA 350

CTTCCTTTTG CCTGCTGGAC TCTCAAAGAA TACTTCAAAT TCGAATGGAC 400

ATTATGAGAC AGCTGTTGAA CCTAAGTTTA ATTCAAGTGG TACTCACTTT 450

TCTAACTTAT CCAAAACAAC TTTCCACTGT TGCTTTCGGA GTGAGCAAGA 500  
 TAGAACTGC TCCTTATGTG CAGACAACAT TGAAGGAAAG ACATTTGTTT 550  
 CNACAGTAAA TTCTTTAGTT TTTCAACAAA TAGATGCAAA CTGGAACATA 600  
 CAGTGCTGGC TAAAAGGAGA CTTAAAATTA TTCATCTGTT ATGTGGAGTC 650  
 5 ATTATTTAAG AATCTATTCA GGAATTATAA CTATAAGGTC CATCTTTTAT 700  
 ATGTTCTGCC TGAAGTGTTA GAAGATTCAC CTCTGGTTCC CAAAAAGGC 750  
 AGTTTTCAGA TGGTTCAGT CAATTGCAGT GTTCATGAAT GTTGTGAATG 800  
 TCTTGTGCCT GTGCCAACAG CCAAACCTCAA CGACACTCTC CTTATGTGTT 850  
 TGAAAATCAC ATCTGGTGGA GTAATTTTCC AGTCACCTCT AATGTCAGTT 900  
 10 CAGCCCATAA ATATGGTGAA GCCTGATCCA CCATTAGGTT TGCATATGGA 950  
 AATCACAGAT GATGGTAATT TAAAGATTTC TTGGTCCAGC CCACCATTGG 1000  
 TACCATTTCC ACTTCAATAT CAAGTGAAAT ATTCAGAGAA TTCTACAACA 1050  
 GTTATCAGAG AAGCTGACAA GATTGTCTCA GCTACATCCC TGCTAGTAGA 1100  
 CAGTATACTT CCTGGGTCTT CGTATGAGGT TCAGGTGAGG GGCAAGAGAC 1150  
 15 TGGATGGCCC AGGAATCTGG AGTGACTGGA GTACTCCTCG TGTCTTTACC 1200  
 ACACAAGATG TCATATACTT TCCACCTAAA ATTCTGACAA GTGTTGGGTC 1250  
 TAATGTTTCT TTTCAGTCA TCTATAAGAA GGAAAACAAG ATTGTTCCCT 1300  
 CAAAAGAGAT TGTTTGGTGG ATGAATTTAG CTGAGAAAAT TCCTCAAAGC 1350  
 CAGTATGATG TTGTGAGTGA TCATGTTAGC AAAGTTACTT TTTTCAATCT 1400  
 20 GAATGAAACC AAACCTCGAG GAAAGTTTAC CTATGATGCA GTGTACTGCT 1450  
 GCAATGAACA TGAATGCCAT CATCGCTATG CTGAATTATA TGTGATTGAT 1500  
 GTCAATATCA ATATCTCATG TGAACTGAT GGGTACTTAA CTAAAATGAC 1550  
 TTGCAGATGG TCAACAGTA CAATCCAGTC ACTGCGGAA AGCACTTTGC 1600  
 AATTGAGGTA TCATAGGAGC AGCCTTTACT GTTCTGATAT TCCATCTATT 1650  
 25 CATCCCATAT CTGAGCCCAA AGATTGCTAT TTGCAGAGTG ATGGTTTTTA 1700  
 TGAATGCATT TTCCAGCCAA TCTTCCTATT ATCTGGCTAC ACAATGTGGA 1750



TTAGGATCAA TCACTCTCTA GGTTCAC TTG ACTCTCCACC AACATGTGTC 1800  
CTTCCTGATT CTGTGGTGAA GCCACTGCCT CCATCCAGTG TGAAAGCAGA 1850  
AATTACTATA AACATTGGAT TATTGAAAAT ATCTTGGGAA AAGCCAGTCT 1900  
TTCCAGAGAA TAACCTTCAA TTCCAGATTC GCTATGGTTT AAGTGGAAAA 1950  
5 GAAGTACAAT GGAAGATGTA TGAGGTTTAT GATGCAAAAT CAAAATCTGT 2000  
CAGTCTCCCA GTTCCAGACT TGTGTGCAGT CTATGCTGTT CAGGTGCGCT 2050  
GTAAGAGGCT AGATGGACTG GGATATTGGA GTAATTGGAG CAATCCAGCC 2100  
TACACAGTTG TCATGGATAT AAAAGTTCCT ATGAGAGGAC CTGAATTTTG 2150  
GAGAATAATT AATGGAGATA CTATGAAAAA GGAGAAAAAT GTCACTTTAC 2200  
10 TTTGGAAGCC CCTGATGAAA AATGACTCAT TGTGCAGTGT TCAGAGATAT 2250  
GTGATAAACC ATCATACTTC CTGCAATGGA ACATGGTCAG AAGATGTGGG 2300  
AAATCACACG AAATTCAC TT TCCTGTGGAC AGAGCAAGCA CATACTGT TA 2350  
CGGTTCTGGC CATCAATTCA ATTGGTGCTT CTGTTGCAAA TTTTAATTTA 2400  
ACCTTTTCAT GGCCTATGAG CAAAGTAAAT ATCGTGCACT CACTCAGTGC 2450  
15 TTATCCTTTA AACAGCAGTT GTGTGATTGT TTCCTGGATA CTATCACCCA 2500  
GTGATTACAA GCTAATGTAT TTTATTATTG AGTGGAAAAA TCTTAATGAA 2550  
GATGGTGAAA TAAAATGGCT TAGAATCTCT TCATCTGT TA AGAAGTATTA 2600  
TATCCATGAT CATTTTATCC CCATTGAGAA GTACCAGTTC AGTCTTTACC 2650  
CAATAITTTAT GGAAGGAGTG GGAAAACCAA AGATAATTAA TAGTTTCACT 2700  
20 CAAGATGATA TTGAAAAACA CCAGAGTGAT GCAGGTTTAT ATGTAATTGT 2750  
GCCAGTAATT ATTCCTCTT CCATCTTATT GCTTGGAACA TTATTAATAT 2800  
CACACCAAAG AATGAAAAAG CTATTTTGGG AAGATGTTCC GAACCCCAAG 2850  
AATTGTTCTT GGGCACAAGG ACTTAATTTT CAGAAGAGAA CGGACATTCT 2900  
TTGAAGTCTA ATCATGATCA CTACAGATGA ACCCAATGTG CCAACTTCCC 2950  
25 AACAGTCTAT AGAGTATTAG AAGATTTT TA CATTTTGAAG AAGGGCCGGA 3000  
ATTC 3004

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3102 base pairs  
 (B) TYPE: Nucleic Acid  
 (C) STRANDEDNESS: Single  
 (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAATTCTCGA GTCGACGGCG GGC GTTAAAG CTCTCGTGGC ATTATCCTTC 50  
 AGTGGGGCTA TTGGACTGAC TTTTCTTATG CTGGGATGTG CCTTAGAGGA 100  
 10 TTATGGGTGT ACTTCTCTGA AGTAAGATGA TTTGTCAAAA ATTCTGTGTG 150  
 GTTTTGTTAC ATTGGGAATT TATTTATGTG ATA ACTGCGT TTA ACTTGTC 200  
 ATATCCAATT ACTCCTTGGA GATTTAAGTT GTCTTGCATG CCACCAAATT 250  
 CAACCTATGA CTA CTTCCTT TTGCCTGCTG GACTCTCAAA GAATACTTCA 300  
 AATTCGAATG GACATTATGA GACAGCTGTT GAACCTAAGT TTAATTCAAG 350  
 15 TGGTACTCAC TTTTCTAACT TATCCAAAAC AACTTTCCAC TGTTGCTTTC 400  
 GGAGTGAGCA AGATAGAAAC TGCTCCTTAT GTGCAGACAA CATTGAAGGA 450  
 AAGACATTTG TTTCAACAGT AAATTCTTTA GTTTTTCAAC AAATAGATGC 500  
 AACTGGAAC ATACAGTGCT GGCTAAAAGG AGACTTAAAA TTATTCATCT 550  
 GTTATGTGGA GTCATTATTT AAGAATCTAT TCAGGAATTA TAACTATAAG 600  
 20 GTCCATCTTT TATATGTTCT GCCTGAAGTG TTAGAAGATT CACCTCTGGT 650  
 TCCCCAAAAA GGCAGTTTTC AGATGGTTCA CTGCAATTGC AGTGTTTCATG 700  
 AATGTTGTGA ATGTCTTGTG CCTGTGCCAA CAGCCAACT CAACGACACT 750  
 CTCCTTATGT GTTTGAAAAT CACATCTGGT GGAGTAATTT TCCAGTCACC 800  
 TCTAATGTCA GTTCAGCCCA TAAATATGGT GAAGCCTGAT CCACCATTAG 850  
 25 GTTTGCATAT GGAAATCACA GATGATGGTA ATTTAAAGAT TTCTTGGTCC 900  
 AGCCCACCAT TGGTACCATT TCCACTTCAA TATCAAGTGA AATATTGAGA 950  
 GAATTCTACA ACAGTTATCA GAGAAGCTGA CAAGATTGTC TCAGCTACAT 1000  
 CCCTGCTAGT AGACAGTATA CTTCTGGGT CTTCTGATGA GGTTCAGGTG 1050

AGGGGCAAGA GACTGGATGG CCCAGGAATC TGGAGTGACT GGAGTACTCC 1100  
TCGTGTCTTT ACCACACAAG ATGTCATATA CTTTCCACCT AAAATTCTGA 1150  
CAAGTGTGG GTCTAATGTT TCTTTTCACT GCATCTATAA GAAGGAAAAC 1200  
AAGATTGTTT CCTCAAAAGA GATTGTTTGG TGGATGAATT TAGCTGAGAA 1250  
5 AATTCCTCAA AGCCAGTATG ATGTTGTGAG TGATCATGTT AGCAAAGTTA 1300  
CTTTTTTCAA TCTGAATGAA ACCAAACCTC GAGGAAAGTT TACCTATGAT 1350  
GCAGTGTACT GCTGCAATGA ACATGAATGC CATCATCGCT ATGCTGAATT 1400  
ATATGTGATT GATGTCAATA TCAATATCTC ATGTGAAACT GATGGGTACT 1450  
TAACTAAAAT GACTTGCAGA TGGTCAACCA GTACAATCCA GTCATTGCG 1500  
10 GAAAGCACTT TGCAATTGAG GTATCATAGG AGCAGCCTTT ACTGTTCTGA 1550  
TATTCCATCT ATTCATCCCA TATCTGAGCC CAAAGATTGC TATTGCAGA 1600  
GTGATGGTTT TTATGAATGC ATTTTCCAGC CAATCTTCCT ATTATCTGGC 1650  
TACACAATGT GGATTAGGAT CAATCACTCT CTAGGTTTAC TTGACTCTCC 1700  
ACCAACATGT GTCCTTCCTG ATTCTGTGGT GAAGCCACTG CCTCCATCCA 1750  
15 GTGTGAAAGC AGAAATTACT ATAAACATTG GATTATTGAA AATATCTTGG 1800  
GAAAAGCCAG TCTTTCCAGA GAATAACCTT CAATTCCAGA TTCGCTATGG 1850  
TTTAAGTGGA AAAGAAGTAC AATGGAAGAT GTATGAGGTT TATGATGCAA 1900  
AATCAAAATC TGTCAGTCTC CCAGTTCCAG ACTTGTGTGC AGTCTATGCT 1950  
GTTCAGGTGC GCTGTAAGAG GCTAGATGGA CTGGGATATT GGAGTAATTG 2000  
20 GAGCAATCCA GCCTACACAG TTGTCATGGA TATAAAAGTT CCTATGAGAG 2050  
GACCTGAATT TTGGAGAATA ATTAATGGAG ATACTATGAA AAAGGAGAAA 2100  
AATGTCACCT TACTTTGGAA GCCCCTGATG AAAAATGACT CATTGTGCAG 2150  
TGTTTCAAGA TATGTGATAA ACCATCATAC TTCCTGCAAT GGAACATGGT 2200  
CAGAAGATGT GGGAAATCAC ACGAAATTCA CTTTCTGTG GACAGAGCAA 2250  
25 GCACATACTG TTACGGTTCT GGCCATCAAT TCAATTGGTG CTTCTGTTGC 2300  
AAATTTTAAT TTAACCTTTT CATGGCCTAT GAGCAAAGTA AATATCGTGC 2350

AGTCACTCAG TGCTTATCCT TTAAACAGCA GTTGTGTGAT TGTTTCCTGG 2400  
 ATACTATCAC CCAGTGATTA CAAGCTAATG TATTTTATTA TTGAGTGGAA 2450  
 AAATCTTAAT GAAGATGGTG AAATAAAATG GCTTAGAATC TCTTCATCTG 2500  
 TTAAGAAGTA TTATATCCAT GATCATTTTA TCCCCATTGA GAAGTACCAG 2550  
 5 TTCAGTCTTT ACCCAATATT TATGGAAGGA GTGGGAAAAC CAAAGATAAT 2600  
 TAATAGTTTC ACTCAAGATG ATATTGAAAA ACACCAGAGT GATGCAGGTT 2650  
 TATATGTAAT TGTGCCAGTA ATTATTTCTT CTTCATCTT ATTGCTTGGA 2700  
 ACATTATTAA TATCACACCA AAGAATGAAA AAGCTATTTT GGGAAGATGT 2750  
 TCCGAACCCC AAGAATTGTT CCTGGGCACA AGGACTTAAT TTTCAGAAGA 2800  
 10 TGTTCCGAAC CCCAAGAATT GTTCCTGGGC ACAAGGACTT AATTTTCAGA 2850  
 AGATGCTTGA AGGCAGCATG TTCGTTAAGA GTCATCACCA CTCCTAATC 2900  
 TCAAGTACCC AGGGACACAA ACACTGCGGA AGGCCACAGG GTCCTCTGCA 2950  
 TAGGAAAACC AGAGACCTTT GTTCACTTGT TTATCTGCTG ACCCTCCCTC 3000  
 CACTATTGTC CTATGACCCT GCCAAATCCC CCTCTGTGAG AAACACCCAA 3050  
 15 GAATGATCAA TAAAAAAAAA AAAAAAAAAA AAAAAAGTCG ACTCGAGAAT 3100  
 TC 3102

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 783 amino acids  
 (B) TYPE: Amino Acid  
 (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

	Met	Met	Cys	Gln	Lys	Phe	Tyr	Val	Val	Leu	Leu	His	Trp	Glu	Phe
	1				5					10				15	
25	Leu	Tyr	Val	Ile	Ala	Ala	Leu	Asn	Leu	Ala	Tyr	Pro	Ile	Ser	Pro
					20					25				30	
	Trp	Lys	Phe	Lys	Leu	Phe	Cys	Gly	Pro	Pro	Asn	Thr	Thr	Asp	Asp
					35					40				45	
30	Ser	Phe	Leu	Ser	Pro	Ala	Gly	Ala	Pro	Asn	Asn	Ala	Ser	Ala	Leu
					50					55				60	

	Lys Gly Ala Ser Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser		
	65	70	75
	Gly Ile Tyr Val Pro Glu Leu Ser Lys Thr Val Phe His Cys Cys		
	80	85	90
5	Phe Gly Asn Glu Gln Gly Gln Asn Cys Ser Ala Leu Thr Asp Asn		
	95	100	105
	Thr Glu Gly Lys Thr Leu Ala Ser Val Val Lys Ala Ser Val Phe		
	110	115	120
10	Arg Gln Leu Gly Val Asn Trp Asp Ile Glu Cys Trp Met Lys Gly		
	125	130	135
	Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro Lys Asn		
	140	145	150
	Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp Leu		
	155	160	165
15	Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser		
	170	175	180
	Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys		
	185	190	195
20	His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met		
	200	205	210
	Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu		
	215	220	225
	Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu		
	230	235	240
25	Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser		
	245	250	255
	Trp Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val		
	260	265	270
30	Lys Tyr Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile		
	275	280	285
	Val Ser Ala Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser		
	290	295	300
	Ser Tyr Glu Val Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly		
	305	310	315

	Val	Trp	Ser	Asp	Trp	Ser	Ser	Pro	Gln	Val	Phe	Thr	Thr	Gln	Asp	
					320					325					330	
	Val	Val	Tyr	Phe	Pro	Pro	Lys	Ile	Leu	Thr	Ser	Val	Gly	Ser	Asn	
					335					340					345	
5	Ala	Ser	Phe	His	Cys	Ile	Tyr	Lys	Asn	Glu	Asn	Gln	Ile	Val	Ser	
					350					355					360	
	Ser	Lys	Gln	Ile	Val	Trp	Trp	Arg	Asn	Leu	Ala	Glu	Lys	Ile	Pro	
					365					370					375	
10	Glu	Ile	Gln	Tyr	Ser	Ile	Val	Ser	Asp	Arg	Val	Ser	Lys	Val	Thr	
					380					385					390	
	Phe	Ser	Asn	Leu	Lys	Ala	Thr	Arg	Pro	Arg	Gly	Lys	Phe	Thr	Tyr	
					395					400					405	
	Asp	Ala	Val	Tyr	Cys	Cys	Asn	Glu	Gln	Ala	Cys	His	His	Arg	Tyr	
					410					415					420	
15	Ala	Glu	Leu	Tyr	Val	Ile	Asp	Val	Asn	Ile	Asn	Ile	Ser	Cys	Glu	
					425					430					435	
	Thr	Asp	Gly	Tyr	Leu	Thr	Lys	Met	Thr	Cys	Arg	Trp	Ser	Pro	Ser	
					440					445					450	
20	Thr	Ile	Gln	Ser	Leu	Val	Gly	Ser	Thr	Val	Gln	Leu	Arg	Tyr	His	
					455					460					465	
	Arg	Cys	Ser	Leu	Tyr	Cys	Pro	Asp	Ser	Pro	Ser	Ile	His	Pro	Thr	
					470					475					480	
	Ser	Glu	Pro	Lys	Thr	Ala	Ser	Tyr	Arg	Glu	Thr	Ala	Phe	Met	Asn	
					485					490					495	
25	Val	Phe	Ser	Ser	Gln	Ser	Phe	Tyr	Tyr	Leu	Ala	Ile	Gln	Cys	Gly	
					500					505					510	
	Phe	Arg	Ile	Asn	His	Ser	Leu	Gly	Ser	Leu	Asp	Ser	Pro	Pro	Thr	
					515					520					525	
30	Cys	Val	Leu	Pro	Asp	Ser	Val	Val	Lys	Pro	Leu	Pro	Pro	Ser	Asn	
					530					535					540	
	Val	Lys	Ala	Glu	Ile	Thr	Val	Asn	Thr	Gly	Leu	Leu	Lys	Val	Ser	
					545					550					555	
	Trp	Glu	Lys	Pro	Val	Phe	Pro	Glu	Asn	Asn	Leu	Gln	Phe	Gln	Ile	
					560					565					570	

	Arg Tyr Gly Leu Ser Gly Lys Glu Ile Gln Trp Lys Thr His Glu	
	575	580 585
	Val Phe Asp Ala Lys Ser Lys Ser Ala Ser Leu Leu Val Ser Asp	
	590	595 600
5	Leu Cys Ala Val Tyr Val Val Gln Val Arg Cys Arg Arg Leu Asp	
	605	610 615
	Gly Leu Gly Tyr Trp Ser Asn Trp Ser Ser Pro Ala Tyr Thr Leu	
	620	625 630
10	Val Met Asp Val Lys Val Pro Met Arg Gly Pro Glu Phe Trp Arg	
	635	640 645
	Lys Met Asp Gly Asp Val Thr Lys Lys Glu Arg Asn Val Thr Leu	
	650	655 660
	Leu Trp Lys Pro Leu Thr Lys Asn Asp Ser Leu Cys Ser Val Arg	
	665	670 675
15	Arg Tyr Val Val Lys His Arg Thr Ala His Asn Gly Thr Trp Ser	
	680	685 690
	Glu Asp Val Gly Asn Arg Thr Asn Leu Thr Phe Leu Trp Thr Glu	
	695	700 705
20	Pro Ala His Thr Val Thr Val Leu Ala Val Asn Ser Leu Gly Ala	
	710	715 720
	Ser Leu Val Asn Phe Asn Leu Thr Phe Ser Trp Pro Met Ser Lys	
	725	730 735
	Val Ser Ala Val Glu Ser Leu Ser Ala Tyr Pro Leu Ser Ser Ser	
	740	745 750
25	Cys Val Ile Leu Ser Trp Thr Leu Ser Pro Asp Asp Tyr Ser Leu	
	755	760 765
	Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly	
	770	775 780
30	Met Lys Trp	
	783	

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- |    |                             |
|----|-----------------------------|
|    | (A) LENGTH: 2868 base pairs |
|    | (B) TYPE: Nucleic Acid      |
| 35 | (C) STRANDEDNESS: Single    |
|    | (D) TOPOLOGY: Linear        |

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGGCCCCCCC TCGAAGTCGA CGGTATCGAT AAGCTTGATA TCGAATTCCG 50  
 GCCGGGACAC AGGTGGGACA CTCTTTTAGT CCTCAATCCC TGGCGCGAGG 100  
 CCACCCAAGG CAACGCAGGA CGCAGGGCGT TTGGGGACCA GGCAGCAGAC 150  
 5 TGGGGCGGTA CCTGCGGAGA GCCACGCAAC TTCTCCAGGC CTCTGACTAC 200  
 TTTGGAAACT GCCCGGGGCT GCGACATCAA CCCCTTAAGT CCCGGAGGCG 250  
 GAAAGAGGGT GGGTTGGTTT GAAAGACACA AGGAAGAAAA ATGTGCTGTG 300  
 GGGCGGGTTA AGTTTCCCAC CCTCTTCCCC CTTCCCGAGC AAATTAGAAA 350  
 CAAAACAAAT AGAAAAGCCA GCCCTCCGGC CAACCAAAGC CCCAAGCGGA 400  
 10 GCCCCAAGCG GAGCCCCAGC CGGAGCACTC CTTTAAAAGG ATTTGCAGCG 450  
 GTGAGGAAAA AACCAGACCC GACCGAGGAA TCGTTCTGCA AATCCAGGTG 500  
 TACACCTCTG AAGAAAGATG ATGTGTCAGA AATTCTATGT GGTTTTGTTA 550  
 CACTGGGAAT TTCTTTATGT GATAGCTGCA CTTAACCTGG CATATCCAAT 600  
 CTCTCCCTGG AAATTTAAGT TGTTTTGTGG ACCACCGAAC ACAACCGATG 650  
 15 ACTCCTTTCT CTCACCTGCT GGAGCCCCAA ACAATGCCTC GGCTTTGAAG 700  
 GGGGCTTCTG AAGCAATTGT TGAAGCTAAA TTTAATTCAA GTGGTATCTA 750  
 CGTTCCTGAG TTATCCAAAA CAGTCTTCCA CTGTTGCTTT GGGAAATGAGC 800  
 AAGGTCAAAA CTGCTCTGCA CTCACAGACA AACTGAAGG GAAGACACTG 850  
 GCTTCAGTAG TGAAGGCTTC AGTTTTTCGC CAGCTAGGTG TAAACTGGGA 900  
 20 CATAGAGTGC TGGATGAAAG GGGACTTGAC ATTATTCATC TGTCATATGG 950  
 AGCCATTACC TAAGAACCCC TTCAAGAATT ATGACTCTAA GGTCCATCTT 1000  
 TTATATGATC TGCCTGAAGT CATAGATGAT TCGCCTCTGC CCCCCTGAA 1050  
 AGACAGCTTT CAGACTGTCC AATGCAACTG CAGTCTTCGG GGATGTGAAT 1100  
 GTCATGTGCC AGTACCCAGA GCCAAACTCA ACTACGCTCT TCTGATGTAT 1150  
 25 TTGGAAATCA CATCTGCCGG TGTGAGTTTT CAGTCACCTC TGATGTCACT 1200  
 GCAGCCCATG CTTGTTGTGA AACCCGATCC ACCCTTAGGT TTGCATATGG 1250



AAGTCACAGA TGATGGTAAT TTAAAGATTT CTTGGGACAG CCAAACAATG 1300  
 GCACCATTTTC CGCTTCAATA TCAGGTGAAA TATTTAGAGA ATTCTACAAT 1350  
 TGTAAGAGAG GCTGCTGAAA TTGTCTCAGC TACATCTCTG CTGGTAGACA 1400  
 GTGTGCTTCC TGGATCTTCA TATGAGGTCC AGGTGAGGAG CAAGAGACTG 1450  
 5 GATGGTTTCAG GAGTCTGGAG TGA CTGGAGT TCACCTCAAG TCTTTACCAC 1500  
 ACAAGATGTT GTGTATTTTC CACCCAAAAT TCTGACTAGT GTTGGATCGA 1550  
 ATGCTTCCTT TCATTGCATC TACAAAAACG AAAACCAGAT TGTCTCCTCA 1600  
 AAACAGATAG TTTGGTGGAG GAATCTAGCT GAGAAAATCC CTGAGATACA 1650  
 GTACAGCATT GTGAGTGACC GAGTTAGCAA AGTTACCTTC TCCAACCTGA 1700  
 10 AAGCCACCAG ACCTCGAGGG AAGTTTACCT ATGACGCAGT GTACTGCTGC 1750  
 AATGAGCAGG CGTGCCATCA CCGCTATGCT GAATTATACG TGATCGATGT 1800  
 CAATATCAAT ATATCATGTG AAAC TGACGG GTACTTAACT AAAATGACTT 1850  
 GCAGATGGTC ACCCAGCACA ATCCAATCAC TAGTGGGAAG CACTGTGCAG 1900  
 CTGAGGTATC ACAGGTGCAG CCTGTATTGT CCTGATAGTC CATCTATTCA 1950  
 15 TCCTACGTCT GAGCCCCAAA CTGCGTCTTA CAGAGAGACG GCTTTTATGA 2000  
 ATGTGTTTTTC CAGCCAATCT TTCTATTATC TGGCTATACA ATGTGGATTC 2050  
 AGGATCAACC ATTCTTTAGG TTCAC TTGAC TCGCCACCAA CGTGTGTCCT 2100  
 TCCTGACTCC GTAGTAAAAC CACTACCTCC ATCTAACGTA AAAGCAGAGA 2150  
 TTACTGTAAA CACTGGATTA TTGAAAGTAT CTTGGGAAAA GCCAGTCTTT 2200  
 20 CCGGAGAATA ACCTTCAATT CCAGATTCGA TATGGCTTAA GTGGAAAAGA 2250  
 AATACAATGG AAGACACATG AGGTATTCTG TGCAAAGTCA AAGTCTGCCA 2300  
 GCCTGCTGGT GTCAGACCTC TGTGCAGTCT ATGTGGTCCA GGTTCTGCTG 2350  
 CGGCGGTTGG ATGGACTAGG ATATTGGAGT AATTGGAGCA GTCCAGCCTA 2400  
 TACGCTTGTC ATGGATGTAA AAGTTCCTAT GAGAGGGCCT GAATTTTGGA 2450  
 25 GAAAAATGGA TGGGGACGTT ACTAAAAAGG AGAGAAATGT CACCTTGCTT 2500  
 TGGAAGCCCC TGACGAAAAA TGA CTCACTG TGTAGTGTGA GGAGGTACGT 2550

GGTGAAGCAT CGTACTGCCC ACAATGGGAC GTGGTCAGAA GATGTGGGAA 2600  
ATCGGACCAA TCTCACTTTC CTGTGGACAG AACCAGCGCA CACTGTTACA 2650  
GTTCTGGCTG TCAATTCCTT CGGCGCTTCC CTTGTGAATT TTAACCTTAC 2700  
CTTCTCATGG CCCATGAGTA AAGTGAGTGC TGTGGAGTCA CTCAGTGCTT 2750  
5 ATCCCCTGAG CAGCAGCTGT GTCATCCTTT CCTGGACACT GTCACCTGAT 2800  
GATTATAGTC TGTTATATCT GGTTATTGAA TGGAAGATCC TTAATGAAGA 2850  
TGATGGAATG AAGTGGCT 2868

## (2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:  
10 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

15 GGGTTAAGTT TCCCACCC 18

## (2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
20 (C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGTGGGAAA CTTAACCC 18

## (2) INFORMATION FOR SEQ ID NO:11:

- 25 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGGATACAGT GGGATCCC 18

## (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 18 base pairs  
    (B) TYPE: Nucleic Acid  
    (C) STRANDEDNESS: Single  
5      (D) TOPOLOGY: Linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:  
  
GCCCCGAGCAC TCCTTTAA 18
- (2) INFORMATION FOR SEQ ID NO:13:
- (i) SEQUENCE CHARACTERISTICS:  
10      (A) LENGTH: 18 base pairs  
        (B) TYPE: Nucleic Acid  
        (C) STRANDEDNESS: Single  
        (D) TOPOLOGY: Linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:  
  
15      TTAAAGGAGT GCTCCCGC 18
- (2) INFORMATION FOR SEQ ID NO:14:
- (i) SEQUENCE CHARACTERISTICS:  
        (A) LENGTH: 18 base pairs  
        (B) TYPE: Nucleic Acid  
20      (C) STRANDEDNESS: Single  
        (D) TOPOLOGY: Linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:  
  
GAGCGGCCCT GTTAGATA 18
- (2) INFORMATION FOR SEQ ID NO:15:
- (i) SEQUENCE CHARACTERISTICS:  
25      (A) LENGTH: 18 base pairs  
        (B) TYPE: Nucleic Acid  
        (C) STRANDEDNESS: Single  
        (D) TOPOLOGY: Linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:  
  
30      GTATACACCT CTGAAGAA 18
- (2) INFORMATION FOR SEQ ID NO:16:
- (i) SEQUENCE CHARACTERISTICS:  
        (A) LENGTH: 18 base pairs  
35      (B) TYPE: Nucleic Acid  
        (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TTCTTCAGAG GTGTACAC 18

(2) INFORMATION FOR SEQ ID NO:17:

- 5 (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 18 base pairs  
    (B) TYPE: Nucleic Acid  
    (C) STRANDEDNESS: Single  
    (D) TOPOLOGY: Linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATGCGAGGCT ACTTCTAT 18

(2) INFORMATION FOR SEQ ID NO:18:

- 15 (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 18 base pairs  
    (B) TYPE: Nucleic Acid  
    (C) STRANDEDNESS: Single  
    (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCTCCCTGG AAATTTAA 18

20 (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 18 base pairs  
    (B) TYPE: Nucleic Acid  
    (C) STRANDEDNESS: Single  
25 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TTAAATTTCC AGGGAGAG 18

(2) INFORMATION FOR SEQ ID NO:20:

- 30 (i) SEQUENCE CHARACTERISTICS:  
    (A) LENGTH: 18 base pairs  
    (B) TYPE: Nucleic Acid  
    (C) STRANDEDNESS: Single  
    (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

ATTGAAGGA GTTAAGCC 18

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AATTTAATTC AAGTGGTA 18

10 (2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TACCAGTTGA ATTAAATT 18

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

25 GTATCACTTC ATAATATA 18

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GATGGTCAGG GTGAACTG 18

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
5 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CAGTTCACCC TGACCATC 18

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:  
10 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

15 GAGGCGAATG TCGGATT 18

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
20 (C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

CTTAAATCTC CAAGGAGT 18

(2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:  
25 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

ACTCCTTGGA GATTTAAG 18

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:  
35 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

AAGTCTTAAG CCAGACTT 18

(2) INFORMATION FOR SEQ ID NO:30:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TCTAAGGCAC ATCCCAGC 18

(2) INFORMATION FOR SEQ ID NO:31:

- 15 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GCTGGGATGT GCCTTAGA 18

20 (2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
25 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CGCAATGAAT TGACCCCC 18

(2) INFORMATION FOR SEQ ID NO:33:

- 30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

TACTTCAGAG AAGTACAC 18

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GTGTACTTCT CTGAAGTA 18

10 (2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GAATCACGGT AACTATCA 18

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

25 CAGCTGTCTC ATAATGTC 18

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GACATTATGA GACAGCTG 18

(2) INFORMATION FOR SEQ ID NO:38:



- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
5 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

TTCGTCAAGC CATCTGAT 18

(2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:  
10 (A) LENGTH: 8 amino acids  
(B) TYPE: Amino Acid  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

His Gln Asn Leu Ser Asp Gly Lys  
15 1 5 8

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: Amino Acid  
20 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

His Gln Asn Ile Ser Asp Gly Lys  
1 5 8

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:  
25 (A) LENGTH: 7 amino acids  
(B) TYPE: Amino Acid  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

30 His Gln Ser Leu Gly Thr Gln  
1 5 7

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:  
35 (A) LENGTH: 8 amino acids  
(B) TYPE: Amino Acid  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Val Ile Ser Ser His Leu Gly Gln  
1 5 8

(2) INFORMATION FOR SEQ ID NO:43:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 11 amino acids  
(B) TYPE: Amino Acid  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

10 Pro Lys Asn Ser Ser Met Ile Ser Asn Thr Pro  
1 5 10 11

(2) INFORMATION FOR SEQ ID NO:44:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 10 amino acids  
15 (B) TYPE: Amino Acid  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
1 5 10

20 (2) INFORMATION FOR SEQ ID NO:45:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 51 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Single  
25 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GTCAGTCTCC CAGTTCCAGA CTTGTGTGCA GTCTATGCTG TTCAGGTGCG 50

C 51

(2) INFORMATION FOR SEQ ID NO:46:

- 30 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7127 base pairs  
(B) TYPE: Nucleic Acid  
(C) STRANDEDNESS: Double  
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TTCGAGCTCG CCCGACATTG ATTATTGACT AGTTATTAAT AGTAATCAAT 50  
 TACGGGGTCA TTAGTTCATA GCCCATATAT GGAGTTCCGC GTTACATAAC 100  
 TTACGGTAAA TGGCCCGCCT GGCTGACCGC CCAACGACCC CCGCCCATTG 150  
 5 ACGTCAATAA TGACGTATGT TCCCATAGTA ACGCCAATAG GGACTTTCCA 200  
 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCCAC TTGGCAGTAC 250  
 ATCAAGTGTA TCATATGCCA AGTACGCCCC CTATTGACGT CAATGACGGT 300  
 AAATGGCCCG CCTGGCATTG TGCCCAGTAC ATGACCTTAT GGGACTTTCC 350  
 TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC 400  
 10 GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGGA 450  
 TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA 500  
 AAATCAACGG GACTTTCCAA AATGTCGTAA CAACTCCGCC CCATTGACGC 550  
 AAATGGGCGG TAGGCGTGTA CGGTGGGAGG TCTATATAAG CAGAGCTCGT 600  
 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT 650  
 15 CCATAGAAGA CACCGGGACC GATCCAGCCT CCGCGGCCCG GAACGGTGCA 700  
 TTGGAACGCG GATTCCCCGT GCCAAGAGTG ACGTAAGTAC CGCCTATAGA 750  
 GTCTATAGGC CCACCCCTT GGCTTCGTGA GAACGCGGCT ACAATTAATA 800  
 CATAACCTTA TGTATCATAC ACATACGATT TAGGTGACAC TATAGAATAA 850  
 CATCCACTTT GCCTTTCTCT CCACAGGTGT CCACTCCCAG GTCCAACTGC 900  
 20 ACCTCGGTTT TATCGATATG CATTGGGGAA CCCTGTGCGG ATTCTTGTGG 950  
 CTTTGGCCCT ATCTTTTCTA TGTCCAAGCT GTGCCCATCC AAAAAGTCCA 1000  
 AGATGACACC AAAACCCTCA TCAAGACAAT TGTCACCAGG ATCAATGACA 1050  
 TTTCACACAC GCAGTCAGTC TCCTCCAAAC AGAAAGTCAC CGGTTTGGAC 1100  
 TTCATTCTTG GGCTCCACCC CATCCTGACC TTATCCAAGA TGGACCAGAC 1150  
 25 ACTGGCAGTC TACCAACAGA TCCTCACCAG TATGCCTTCC AGAAACGTGA 1200  
 TCCAAATATC CAACGACCTG GAGAACCTCC GGGATCTTCT TCACGTGCTG 1250

GCCTTCTCTA AGAGCTGCCA CTTGCCCTGG GCCAGTGGCC TGGAGACCTT 1300  
 GGACAGCCTG GGGGGTGTCC TGGAAGCTTC AGGCTACTCC ACAGAGGTGG 1350  
 TGGCCCTGAG CAGGCTGCAG GGGTCTCTGC AGGACATGCT GTGGCAGCTG 1400  
 GACCTCAGCC CTGGGTGCGG GGTCAACGAC AAAACTCACA CATGCCCACC 1450  
 5 GTGCCCAGCA CCTGAACTCC TGGGGGGACC GTCAGTCTTC CTCTTCCCCC 1500  
 CAAAACCCAA GGACACCCTC ATGATCTCCC GGACCCCTGA GGTCACATGC 1550  
 GTGGTGGTGG ACGTGAGCCA CGAAGACCCT GAGGTCAAGT TCAACTGGTA 1600  
 CGTGACGGC GTGGAGGTGC ATAATGCCAA GACAAAGCCG CGGGAGGAGC 1650  
 AGTACAACAG CACGTACCGT GTGGTCAGCG TCCTACCGT CCTGCACCAG 1700  
 10 GACTGGCTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAAGCCCT 1750  
 CCCAGCCCCC ATCGAGAAAA CCATCTCCAA AGCCAAAGGG CAGCCCCGAG 1800  
 AACCACAGGT GTACACCCTG CCCCATCCC GGAAGAGAT GACCAAGAAC 1850  
 CAGGTCAGCC TGACCTGCCT GGTCAAAGGC TTCTATCCCA GCGACATCGC 1900  
 CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GAACAACTAC AAGACCACGC 1950  
 15 CTCCCGTGCT GGACTCCGAC GGCTCCTTCT TCCTCTACAG CAAGCTCACC 2000  
 GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCCGTGAT 2050  
 GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC 2100  
 CGGGTAAATG AGTGCGACGG CCCTAGAGTC GACCTGCAGA AGCTTCTAGA 2150  
 GTCGACCTGC AGAAGCTTGG CCGCCATGGC CCAACTTGTT TATTGCAGCT 2200  
 20 TATAATGGTT ACAAATAAAG CAATAGCATC ACAAATTTCA CAAATAAAGC 2250  
 ATTTTTTTCA CTGCATTCTA GTTGTGGTTT GTCCAACTC ATCAATGTAT 2300  
 CTTATCATGT CTGGATCGAT CGGGAATTAA TTCGGCGCAG CACCATGGCC 2350  
 TGAAATAACC TCTGAAAGAG GAACTTGGTT AGGTACCTTC TGAGGCGGAA 2400  
 AGAACCAGCT GTGGAATGTG TGTCAGTTAG GGTGTGGAAA GTCCCCAGGC 2450  
 25 TCCCCAGCAG GCAGAAGTAT GCAAAGCATG CATCTCAATT AGTCAGCAAC 2500  
 CAGGTGTGGA AAGTCCCCAG GCTCCCCAGC AGGCAGAAGT ATGCAAAGCA 2550

TGCATCTCAA TTAGTCAGCA ACCATAGTCC CGCCCCTAAC TCCGCCCATC 2600  
CCGCCCTTAA CTCCGCCAG TTCCGCCAT TCTCCGCCCC ATGGCTGACT 2650  
AATTTTTTTT ATTTATGCAG AGGCCGAGGC CGCCTCGGCC TCTGAGCTAT 2700  
TCCAGAAGTA GTGAGGAGGC TTTTTTGGAG GCCTAGGCTT TTGCAAAAAG 2750  
5 CTGTTAATTC GAACACGCAG ATGCAGTCGG GCGGGCGCGG TCCCAGGTCC 2800  
ACTTCGCATA TTAAGGTGAC GCGTGTGGCC TCGAACACCG AGCGACCCTG 2850  
CAGCGACCCG CTTAACAGCG TCAACAGCGT GCCGCAGATC TGATCAAGAG 2900  
ACAGGATGAG GATCGTTTCG CATGATTGAA CAAGATGGAT TGCACGCAGG 2950  
TTCTCCGGCC GCTTGGGTGG AGAGGCTATT CGGCTATGAC TGGGCACAAC 3000  
10 AGACAATCGG CTGCTCTGAT GCCGCCGTGT TCCGGCTGTC AGCGCAGGGG 3050  
CGCCCGGTTT TTTTGTCAA GACCGACCTG TCCGGTGCCC TGAATGAACT 3100  
GCAGGACGAG GCAGCGCGGC TATCGTGGCT GGCCACGACG GCGGTTCTCT 3150  
GCGCAGCTGT GCTCGACGTT GTCCTGAAG CGGGAAGGGA CTGGCTGCTA 3200  
TTGGGCGAAG TGCCGGGGCA GGATCTCTCTG TCATCTCACC TTGCTCCTGC 3250  
15 CGAGAAAGTA TCCATCATGG CTGATGCAAT GCGGCGGCTG CATACGCTTG 3300  
ATCCGGCTAC CTGCCCATTG GACCACCAAG CGAAACATCG CATCGAGCGA 3350  
GCACGTACTC GGATGGAAGC CGGTCTTGTC GATCAGGATG ATCTGGACGA 3400  
AGAGCATCAG GGGCTCGCGC CAGCCGAAC TTTCCGAGG CTCAAGGCGC 3450  
GCATGCCCCA CGGCGAGGAT CTCGTCGTGA CCCATGGCGA TGCCTGCTTG 3500  
20 CCGAATATCA TGGTGGAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG 3550  
CCGGCTGGGT GTGGCGGACC GCTATCAGGA CATAGCGTTG GCTACCCGTG 3600  
ATATTGCTGA AGAGCTTGGC GGCGAATGGG CTGACCGCTT CCTCGTGCTT 3650  
TACGGTATCG CCGCTCCCGA TTCGCAGCGC ATCGCCTTCT ATCGCCTTCT 3700  
TGACGAGTTC TTCTGAGCGG GACTCTGGGG TTCGAAATGA CCGACCAAGC 3750  
25 GACGCCCAAC CTGCCATCAC GAGATTTCGA TTCCACCGCC GCCTTCTATG 3800  
AAAGGTTGGG CTTCCGAATC GTTTTCCGGG ACGCCGGCTG GATGATCCTC 3850

CAGCGCGGGG ATCTCATGCT GGAGTTCTTC GCCCAGCCCG GGAGATGGGG 3900  
GAGGCTAACT GAAACACGGA AGGAGACAAT ACCGGAAGGA ACCCGCGCTA 3950  
TGACGGCAAT AAAAAGACAG AATAAACGC ACGGGTGTTG GGTCTTTGT 4000  
TCATAACGC GGGGTTCGGT CCCAGGGCTG GCACTCTGTC GATACCCAC 4050  
5 CGAGACCCCA TTGGGGCCAA TACGCGCG TTTCTTCCTT TCCCCACCC 4100  
CAACCCCAA GTTCGGGTGA AGGCCCAGGG CTCGCAGCCA ACGTCGGGGC 4150  
GGCAAGCCCG CCATAGCCAC GGGCCCCGTG GGTTAGGGAC GGGGTCCCCC 4200  
ATGGGAATG GTTTATGGT CGTGGGGT ATTCTTTTG GCGTTGCGTG 4250  
GGGTCAGGTC CACGACTGGA CTGAGCAGAC AGACCCATGG TTTTGGATG 4300  
10 GCCTGGGCAT GGACCGCATG TACTGGCGCG ACACGAACAC CGGGCGTCTG 4350  
TGGCTGCCAA ACACCCCGA CCCCCAAAA CCACCGCGCG GATTCTGGC 4400  
GCCGCCGAC GAACTAAACC TGA CTACGGC ATCTCTGCC CTTCTTCGCT 4450  
GGTACGAGGA GCGCTTTTGT TTTGTATTGG TCACCACGGC CGAGTTTCCG 4500  
CGGGACCCCG GCCAGGGCAC CTGTCTACG AGTTGCATGA TAAAGAAGAC 4550  
15 AGTCATAAGT GCGGCGACGA TAGTCATGCC CCGCGCCAC CGGAAGGAGC 4600  
TGA CTGGGT GAAGGCTCTC AAGGGCATCG GTCGAGCGGC CGCATCAAAG 4650  
CAACCATAGT ACGCGCCCTG TAGCGGCGCA TTAAGCGCGG CGGGTGTGGT 4700  
GGTTACGCGC AGCGTGACCG CTACACTTGC CAGCGCCCTA GCGCCGCTC 4750  
CTTTCGCTTT CTTCCCTTCC TTTCTCGCCA CGTTCGCCG CTTTCCCCGT 4800  
20 CAAGCTCTAA ATCGGGGGCT CCCTTTAGGG TTCCGATTTA GTGCTTTACG 4850  
GCACCTCGAC CCAAAAAAC TTGATTGGG TGATGGTTCA CGTAGTGGGC 4900  
CATCGCCCTG ATAGACGGT TTTGCGCCTT TGACGTGGA GTCCACGTT 4950  
TTTAATAGTG GACTCTTGTT CCAAACCTGA ACAACACTCA ACCCTATCTC 5000  
GGGCTATTCT TTTGATTAT AAGGGATTTT GCCGATTTG GCCTATTGGT 5050  
25 TAAAAAATGA GCTGATTAA CAAAAATTA ACGCGAATTT TAACAAAATA 5100  
TTAACGTTTA CAATTTTATG GTGCAGGCCT CGTGATACGC CTATTTTAT 5150

AGGTTAATGT CATGATAATA ATGGTTTCTT AGACGTCAGG TGGCACTTTT 5200  
CGGGGAAAATG TGC GCGGAAC CCCTATTTGT TTATTTTCT AAATACATTC 5250  
AAATATGTAT CCGCTCATGA GACAATAACC CTGATAAATG CTTCAATAAT 5300  
ATTGAAAAAG GAAGAGTATG AGTATTCAAC ATTTCCGTGT CGCCCTTATT 5350  
5 CCCTTTTTTG CGGCATTTTG CCTTCCTGTT TTTGCTCACC CAGAAACGCT 5400  
GGTGAAAGTA AAAGATGCTG AAGATCAGTT GGGTGACGA GTGGGTTACA 5450  
TCGAACTGGA TCTCAACAGC GGTAAGATCC TTGAGAGTTT TCGCCCCGAA 5500  
GAACGTTTTT CAATGATGAG CACTTTTAAA GTTCTGCTAT GTGGCGCGGT 5550  
ATTATCCCGT GATGACGCCG GGCAAGAGCA ACTCGGTCGC CGCATACT 5600  
10 ATTCTCAGAA TGACTTGTT GAGTACTCAC CAGTCACAGA AAAGCATCTT 5650  
ACGGATGGCA TGACAGTAAG AGAATTATGC AGTGCTGCCA TAACCATGAG 5700  
TGATAACACT GCGGCCAACT TACTTCTGAC AACGATCGGA GGACCGAAGG 5750  
AGCTAACC GC TTTTTCAC AACATGGGG ATCATGTAAC TCGCCTTGAT 5800  
CGTTGGGAAC CGGAGCTGAA TGAAGCCATA CCAAACGACG AGCGTGACAC 5850  
15 CACGATGCCA GCAGCAATGG CAACAACGTT GCGCAAACTA TTAAGTGGCG 5900  
AACTACTTAC TCTAGCTTCC CGGCAACAAT TAATAGACTG GATGGAGGCG 5950  
GATAAAGTTG CAGGACCACT TCTGCGCTCG GCCCTTCCGG CTGGCTGGTT 6000  
TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGGTCTCGC GGTATCATTG 6050  
CAGCACTGGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG 6100  
20 ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGAT 6150  
AGGTGCCTCA CTGATTAAGC ATTGGTAACT GTCAGACCAA GTTTACTCAT 6200  
ATATACTTTA GATTGATTTA AACTTCATT TTTAATTTAA AAGGATCTAG 6250  
GTGAAGATCC TTTTGTATA TCTCATGACC AAAATCCCTT AACGTGAGTT 6300  
TTCGTTCCAC TGAGCGTCAG ACCCCGTAGA AAAGATCAAA GGATCTTCTT 6350  
25 GAGATCCTTT TTTTCTGCGC GTAATCTGCT GCTTGCAAAC AAAAAACCA 6400  
CCGCTACCAG CGGTGGTTTG TTTGCCGGAT CAAGAGCTAC CAACTCTTTT 6450

TCCGAAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAAT ACTGTCCTTC 6500

TAGTGTAGCC GTAGTTAGGC CACCACTTCA AGAACTCTGT AGCACCGCCT 6550

ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA 6600

TAAGTCGTGT CTTACCGGGT TGGACTCAAG ACGATAGTTA CCGGATAAGG 6650

5 CGCAGCGGTC GGGCTGAACG GGGGGTTTCGT GCACACAGCC CAGCTTGGAG 6700

CGAACGACCT ACACCGAACT GAGATACCTA CAGCGTGAGC ATTGAGAAAAG 6750

CGCCACGCTT CCCGAAGGGA GAAAGGCGGA CAGGTATCCG GTAAGCGGCA 6800

GGGTCGGAAC AGGAGAGCGC ACGAGGGAGC TTCCAGGGGG AAACGCCTGG 6850

TATCTTTATA GTCCTGTCGG GTTTCGCCAC CTCTGACTTG AGCGTCGATT 6900

10 TTTGTGATGC TCGTCAGGGG GCGGAGCCT ATGGAAAAAC GCCAGCTGGC 6950

ACGACAGGTT TCCCGACTGG AAAGCGGGCA GTGAGCGCAA CGCAATTAAT 7000

GTGAGTTACC TCACTCATTA GGCACCCAG GCTTTACACT TTATGCTTCC 7050

GGCTCGTATG TTGTGTGGAA TTGTGAGCGG ATAACAATTT CACACAGGAA 7100

ACAGCTATGA CCATGATTAC GAATTAA 7127

15 (2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 397 amino acids
- (B) TYPE: Amino Acid
- (D) TOPOLOGY: Linear

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Met	His	Trp	Gly	Thr	Leu	Cys	Gly	Phe	Leu	Trp	Leu	Trp	Pro	Tyr
1				5					10					15
Leu	Phe	Tyr	Val	Gln	Ala	Val	Pro	Ile	Gln	Lys	Val	Gln	Asp	Asp
			20						25					30
25	Thr	Lys	Thr	Leu	Ile	Lys	Thr	Ile	Val	Thr	Arg	Ile	Asn	Asp
			35						40					45
Ser	His	Thr	Gln	Ser	Val	Ser	Ser	Lys	Gln	Lys	Val	Thr	Gly	Leu
			50						55					60
30	Asp	Phe	Ile	Pro	Gly	Leu	His	Pro	Ile	Leu	Thr	Leu	Ser	Lys
			65						70					75



	Asp	Gln	Thr	Leu	Ala	Val	Tyr	Gln	Gln	Ile	Leu	Thr	Ser	Met	Pro	
																80 85 90
	Ser	Arg	Asn	Val	Ile	Gln	Ile	Ser	Asn	Asp	Leu	Glu	Asn	Leu	Arg	
																95 100 105
5	Asp	Leu	Leu	His	Val	Leu	Ala	Phe	Ser	Lys	Ser	Cys	His	Leu	Pro	
																110 115 120
	Trp	Ala	Ser	Gly	Leu	Glu	Thr	Leu	Asp	Ser	Leu	Gly	Gly	Val	Leu	
																125 130 135
	Glu	Ala	Ser	Gly	Tyr	Ser	Thr	Glu	Val	Val	Ala	Leu	Ser	Arg	Leu	
10																140 145 150
	Gln	Gly	Ser	Leu	Gln	Asp	Met	Leu	Trp	Gln	Leu	Asp	Leu	Ser	Pro	
																155 160 165
	Gly	Cys	Gly	Val	Thr	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	
																170 175 180
15	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	
																185 190 195
	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	
																200 205 210
	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	
20																215 220 225
	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	
																230 235 240
	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	
																245 250 255
25	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	
																260 265 270
	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	
																275 280 285
	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	
30																290 295 300
	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	
																305 310 315
	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	
																320 325 330

	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro
					335					340					345
	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu
					350					355					360
5	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys
					365					370					375
	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser
					380					385					390
	Leu	Ser	Leu	Ser	Pro	Gly	Lys								
10					395		397								

## (2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 249 amino acids
  - (B) TYPE: Amino Acid
  - (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

	Glu	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys	Pro	Gly
	1				5					10					15
	Ala	Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr
20					20					25					30
	Gly	Tyr	Tyr	Met	Tyr	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu
					35					40					45
	Glu	Trp	Met	Gly	Trp	Ile	Asn	Pro	Asn	Ser	Gly	Gly	Thr	Asn	Tyr
					50					55					60
25	Ala	Gln	Lys	Phe	Gln	Gly	Arg	Val	Thr	Met	Thr	Arg	Asp	Thr	Ser
					65					70					75
	Ile	Gly	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Arg	Leu	Ser	Ser	Asp	Asp
					80					85					90
	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Asp	Arg	Tyr	Tyr	Gly	Ser	Ser
30					95					100					105
	Ala	Tyr	His	Arg	Gly	Ser	Tyr	Tyr	Met	Asp	Val	Trp	Gly	Arg	Gly
					110					115					120
	Thr	Leu	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Thr	Gly	Gly	Gly
					125					130					135

Gly Ser Gly Gly Gly Gly Ser Ser Glu Leu Thr Gln Asp Pro Ala  
 140 145 150  
 Val Ser Val Ala Leu Gly Gln Thr Val Arg Ile Thr Cys Gln Gly  
 155 160 165  
 5 Asp Ser Leu Arg Ser Tyr Tyr Ala Ser Trp Tyr Gln Gln Lys Pro  
 170 175 180  
 Gly Gln Ala Pro Val Leu Val Ile Tyr Gly Lys Asn Asn Arg Pro  
 185 190 195  
 10 Ser Gly Ile Pro Asp Arg Phe Ser Gly Ser Ser Ser Gly Asn Thr  
 200 205 210  
 Ala Ser Leu Thr Ile Thr Gly Ala Gln Ala Glu Asp Glu Ala Asp  
 215 220 225  
 Tyr Tyr Cys Asn Ser Arg Asp Ser Ser Gly Asn His Val Val Phe  
 230 235 240  
 15 Gly Gly Gly Thr Lys Leu Thr Val Leu  
 245 249

## (2) INFORMATION FOR SEQ ID NO:49:

## (i) SEQUENCE CHARACTERISTICS:

- 20 (A) LENGTH: 250 amino acids  
 (B) TYPE: Amino Acid  
 (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly  
 1 5 10 15  
 25 Glu Ser Leu Lys Ile Ser Cys Gln Gly Ser Gly Phe Thr Phe Ser  
 20 25 30  
 Ser Tyr Lys Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu  
 35 40 45  
 30 Glu Trp Met Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr  
 50 55 60  
 Ala Gln Lys Phe Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser  
 65 70 75  
 Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp  
 80 85 90

	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Asp	Arg	Val	Val	Val	Pro	Ala	
						95				100					105	
	Thr	Ser	Leu	Arg	Gly	Gly	Met	Asp	Val	Trp	Gly	Gln	Gly	Thr	Thr	
					110					115					120	
5	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	
					125					130					135	
	Gly	Gly	Gly	Gly	Ser	Gln	Ser	Val	Leu	Thr	Gln	Pro	Ala	Ser	Val	
					140					145					150	
	Ser	Gly	Ser	Pro	Gly	Gln	Ser	Ile	Thr	Ile	Ser	Cys	Thr	Gly	Thr	
10					155					160					165	
	Ser	Ser	Asp	Val	Gly	Gly	Tyr	Asn	Tyr	Val	Ser	Trp	Tyr	Gln	Gln	
					170					175					180	
	His	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Met	Ile	Tyr	Glu	Gly	Ser	Lys	
					185					190					195	
15	Arg	Pro	Ser	Gly	Val	Ser	Asn	Arg	Phe	Ser	Gly	Ser	Lys	Ser	Gly	
					200					205					210	
	Ser	Thr	Ala	Ser	Leu	Thr	Ile	Ser	Gly	Leu	Gln	Ala	Glu	Asp	Glu	
					215					220					225	
	Ala	Asp	Tyr	Tyr	Cys	Ser	Ser	Tyr	Thr	Thr	Arg	Ser	Thr	Arg	Val	
20					230					235					240	
	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Thr	Val	Leu						
					245					250						

## (2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
- 25 (A) LENGTH: 241 amino acids
- (B) TYPE: Amino Acid
- (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

30	Gln	Val	Arg	Leu	Gln	Gln	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	
	1				5					10					15	
	Arg	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Asp	
					20					25					30	
	Asp	Tyr	Ala	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	
					35					40					45	

	Glu Trp Val Ser Gly Met Thr Trp Asn Ser Gly Ser Ile Gly Tyr	
	50	55 60
	Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala	
	65	70 75
5	Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp	
	80	85 90
	Thr Ala Val Tyr Tyr Cys Ala Arg Glu Pro His Asn Thr Asp Ala	
	95	100 105
10	Phe Asp Ile Trp Gly Arg Gly Thr Leu Val Thr Val Ser Ser Gly	
	110	115 120
	Gly Gly Gly Pro Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp	
	125	130 135
	Val Val Met Thr Gln Ser Pro Ser Phe Leu Ser Ala Phe Val Gly	
	140	145 150
15	Asp Thr Ile Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Tyr Asn	
	155	160 165
	Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu	
	170	175 180
	Leu Ile Tyr Ala Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg	
20	185	190 195
	Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser	
	200	205 210
	Ser Leu Gln Pro Glu Asp Phe Gly Thr Tyr Tyr Cys Gln Gln Leu	
	215	220 225
25	Ile Ser Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile	
	230	235 240

Lys

241

## (2) INFORMATION FOR SEQ ID NO:51:

30

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 894 amino acids
- (B) TYPE: Amino Acid
- (D) TOPOLOGY: Linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

	Met Met Cys Gln Lys Phe Tyr Val Val Leu Leu His Trp Glu Phe	
	1 5 10 15	
	Leu Tyr Val Ile Ala Ala Leu Asn Leu Ala Tyr Pro Ile Ser Pro	
	20 25 30	
5	Trp Lys Phe Lys Leu Phe Cys Gly Pro Pro Asn Thr Thr Asp Asp	
	35 40 45	
	Ser Phe Leu Ser Pro Ala Gly Ala Pro Asn Asn Ala Ser Ala Leu	
	50 55 60	
10	Lys Gly Ala Ser Glu Ala Ile Val Glu Ala Lys Phe Asn Ser Ser	
	65 70 75	
	Gly Ile Tyr Val Pro Glu Leu Ser Lys Thr Val Phe His Cys Cys	
	80 85 90	
	Phe Gly Asn Glu Gln Gly Gln Asn Cys Ser Ala Leu Thr Asp Asn	
	95 100 105	
15	Thr Glu Gly Lys Thr Leu Ala Ser Val Val Lys Ala Ser Val Phe	
	110 115 120	
	Arg Gln Leu Gly Val Asn Trp Asp Ile Glu Cys Trp Met Lys Gly	
	125 130 135	
	Asp Leu Thr Leu Phe Ile Cys His Met Glu Pro Leu Pro Lys Asn	
20	140 145 150	
	Pro Phe Lys Asn Tyr Asp Ser Lys Val His Leu Leu Tyr Asp Leu	
	155 160 165	
	Pro Glu Val Ile Asp Asp Ser Pro Leu Pro Pro Leu Lys Asp Ser	
	170 175 180	
25	Phe Gln Thr Val Gln Cys Asn Cys Ser Leu Arg Gly Cys Glu Cys	
	185 190 195	
	His Val Pro Val Pro Arg Ala Lys Leu Asn Tyr Ala Leu Leu Met	
	200 205 210	
	Tyr Leu Glu Ile Thr Ser Ala Gly Val Ser Phe Gln Ser Pro Leu	
30	215 220 225	
	Met Ser Leu Gln Pro Met Leu Val Val Lys Pro Asp Pro Pro Leu	
	230 235 240	
	Gly Leu His Met Glu Val Thr Asp Asp Gly Asn Leu Lys Ile Ser	
	245 250 255	

	Trp Asp Ser Gln Thr Met Ala Pro Phe Pro Leu Gln Tyr Gln Val	
	260	265 270
	Lys Tyr Leu Glu Asn Ser Thr Ile Val Arg Glu Ala Ala Glu Ile	
	275	280 285
5	Val Ser Ala Thr Ser Leu Leu Val Asp Ser Val Leu Pro Gly Ser	
	290	295 300
	Ser Tyr Glu Val Gln Val Arg Ser Lys Arg Leu Asp Gly Ser Gly	
	305	310 315
10	Val Trp Ser Asp Trp Ser Ser Pro Gln Val Phe Thr Thr Gln Asp	
	320	325 330
	Val Val Tyr Phe Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn	
	335	340 345
	Ala Ser Phe His Cys Ile Tyr Lys Asn Glu Asn Gln Ile Ile Ser	
	350	355 360
15	Ser Lys Gln Ile Val Trp Trp Arg Asn Leu Ala Glu Lys Ile Pro	
	365	370 375
	Glu Ile Gln Tyr Ser Ile Val Ser Asp Arg Val Ser Lys Val Thr	
	380	385 390
20	Phe Ser Asn Leu Lys Ala Thr Arg Pro Arg Gly Lys Phe Thr Tyr	
	395	400 405
	Asp Ala Val Tyr Cys Cys Asn Glu Gln Ala Cys His His Arg Tyr	
	410	415 420
	Ala Glu Leu Tyr Val Ile Asp Val Asn Ile Asn Ile Ser Cys Glu	
	425	430 435
25	Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg Trp Ser Pro Ser	
	440	445 450
	Thr Ile Gln Ser Leu Val Gly Ser Thr Val Gln Leu Arg Tyr His	
	455	460 465
30	Arg Arg Ser Leu Tyr Cys Pro Asp Ser Pro Ser Ile His Pro Thr	
	470	475 480
	Ser Glu Pro Lys Asn Cys Val Leu Gln Arg Asp Gly Phe Tyr Glu	
	485	490 495
	Cys Val Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp	
	500	505 510

	Ile	Arg	Ile	Asn	His	Ser	Leu	Gly	Ser	Leu	Asp	Ser	Pro	Pro	Thr
					515					520					525
	Cys	Val	Leu	Pro	Asp	Ser	Val	Val	Lys	Pro	Leu	Pro	Pro	Ser	Asn
					530					535					540
5	Val	Lys	Ala	Glu	Ile	Thr	Val	Asn	Thr	Gly	Leu	Leu	Lys	Val	Ser
					545					550					555
	Trp	Glu	Lys	Pro	Val	Phe	Pro	Glu	Asn	Asn	Leu	Gln	Phe	Gln	Ile
					560					565					570
10	Arg	Tyr	Gly	Leu	Ser	Gly	Lys	Glu	Ile	Gln	Trp	Lys	Thr	His	Glu
					575					580					585
	Val	Phe	Asp	Ala	Lys	Ser	Lys	Ser	Ala	Ser	Leu	Leu	Val	Ser	Asp
					590					595					600
	Leu	Cys	Ala	Val	Tyr	Val	Val	Gln	Val	Arg	Cys	Arg	Arg	Leu	Asp
					605					610					615
15	Gly	Leu	Gly	Tyr	Trp	Ser	Asn	Trp	Ser	Ser	Pro	Ala	Tyr	Thr	Leu
					620					625					630
	Val	Met	Asp	Val	Lys	Val	Pro	Met	Arg	Gly	Pro	Glu	Phe	Trp	Arg
					635					640					645
20	Lys	Met	Asp	Gly	Asp	Val	Thr	Lys	Lys	Glu	Arg	Asn	Val	Thr	Leu
					650					655					660
	Leu	Trp	Lys	Pro	Leu	Thr	Lys	Asn	Asp	Ser	Leu	Cys	Ser	Val	Arg
					665					670					675
	Arg	Tyr	Val	Val	Lys	His	Arg	Thr	Ala	His	Asn	Gly	Thr	Trp	Ser
					680					685					690
25	Glu	Asp	Val	Gly	Asn	Arg	Thr	Asn	Leu	Thr	Phe	Leu	Trp	Thr	Glu
					695					700					705
	Pro	Ala	His	Thr	Val	Thr	Val	Leu	Ala	Val	Asn	Ser	Leu	Gly	Ala
					710					715					720
30	Ser	Leu	Val	Asn	Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	Met	Ser	Lys
					725					730					735
	Val	Ser	Ala	Val	Glu	Ser	Leu	Ser	Ala	Tyr	Pro	Leu	Ser	Ser	Ser
					740					745					750
	Cys	Val	Ile	Leu	Ser	Trp	Thr	Leu	Ser	Pro	Asp	Asp	Tyr	Ser	Leu
					755					760					765



	Leu Tyr Leu Val Ile Glu Trp Lys Ile Leu Asn Glu Asp Asp Gly	
	770	780
	Met Lys Trp Leu Arg Ile Pro Ser Asn Val Lys Lys Phe Tyr Ile	
	785	795
5	His Asp Asn Phe Ile Pro Ile Glu Lys Tyr Gln Phe Ser Leu Tyr	
	800	810
	Pro Val Phe Met Glu Gly Val Gly Lys Pro Lys Ile Ile Asn Gly	
	815	825
10	Phe Thr Lys Asp Ala Ile Asp Lys Gln Gln Asn Asp Ala Gly Leu	
	830	840
	Tyr Val Ile Val Pro Ile Ile Ile Ser Ser Cys Val Leu Leu Leu	
	845	855
	Gly Thr Leu Leu Ile Ser His Gln Arg Met Lys Lys Leu Phe Trp	
	860	870
15	Asp Asp Val Pro Asn Pro Lys Asn Cys Ser Trp Ala Gln Gly Leu	
	875	885
	Asn Phe Gln Lys Arg Thr Asp Thr Leu	
	890	894

## WHAT IS CLAIMED IS:

1. Isolated WSX receptor.
2. The WSX receptor of claim 1 comprising an amino acid sequence selected from the group consisting of: (a) the amino acid sequence of mature human WSX receptor variant 6.4 shown in Figs 2A-B; (b)  
5 the amino acid sequence of mature human WSX receptor variant 12.1 shown in Figs. 2A-B; and (c) the amino acid sequence of mature human WSX receptor variant 13.2 shown in Figs 2A-B.
3. The WSX receptor of claim 2 which is mature human WSX receptor variant 13.2.
4. The WSX receptor of claim 1 which is WSX receptor extracellular domain (ECD).
5. The WSX receptor ECD of claim 4 which is conjugated with, or fused to, a molecule which  
10 increases the serum half-life thereof.
6. The WSX receptor ECD of claim 5 which is conjugated with polyethylene glycol (PEG).
7. A composition comprising the WSX receptor ECD of claim 4 and a physiologically acceptable carrier.
8. The composition of claim 7 further comprising WSX ligand.
9. The WSX receptor of claim 1 which is chimeric WSX receptor.  
15
10. The chimeric WSX receptor of claim 9 comprising a WSX receptor amino acid sequence fused to an immunoglobulin sequence.
11. The chimeric WSX receptor of claim 10 comprising a fusion of a WSX receptor extracellular domain sequence to an immunoglobulin constant domain sequence.
12. The chimeric WSX receptor of claim 11 wherein said constant domain sequence is that of an  
20 immunoglobulin heavy chain.
13. A method for identifying a molecule which binds to the WSX receptor comprising exposing the WSX receptor to a molecule suspected of binding thereto and determining binding of the molecule to the WSX receptor.
14. A method for identifying a molecule which activates the WSX receptor comprising exposing  
25 the WSX receptor to a molecule suspected of being capable of activating the WSX receptor and measuring activation of the WSX receptor.
15. A method for purifying a molecule which binds to the WSX receptor comprising adsorbing the molecule to WSX receptor immobilized on a solid phase and recovering the molecule from the immobilized  
30 WSX receptor.
16. An antibody that specifically binds to the WSX receptor of claim 1.
17. The antibody of claim 16 which is an agonist antibody.
18. The antibody of claim 17 which has an IC<sub>50</sub> in a KIRA ELISA of about 0.5µg/ml or less.
19. The antibody of claim 16 which is a neutralizing antibody.

20. The antibody of claim 16 which is a human or humanized antibody.
21. The antibody of claim 16 which is an antibody fragment.
22. The antibody fragment of claim 21 which is an F(ab')<sub>2</sub>.
23. A composition comprising the antibody of claim 16 and a physiologically acceptable carrier.
- 5 24. The composition of claim 23 further comprising a cytokine.
25. A method for activating the WSX receptor comprising exposing the WSX receptor to an amount of the antibody of claim 17 which is effective for activating the WSX receptor.
26. A method for enhancing proliferation or differentiation of a cell comprising the WSX receptor comprising exposing the cell to an amount of the antibody of claim 17 which is effective for enhancing proliferation or differentiation of the cell.
- 10 27. The method of claim 26 wherein the cell is a CD34+ cell.
28. A method for determining the presence of a WSX receptor comprising exposing a test sample suspected of containing the WSX receptor to the antibody of claim 16 and determining binding of said antibody to the test sample.
- 15 29. An isolated nucleic acid molecule encoding the WSX receptor of claim 1.
30. An isolated nucleic acid molecule encoding the WSX receptor ECD of claim 4.
31. An isolated nucleic acid molecule encoding the chimeric WSX receptor of claim 9.
32. The isolated nucleic acid molecule of any one of claims 29-31 further comprising a promoter operably linked to the nucleic acid molecule.
- 20 33. An expression vector comprising the nucleic acid molecule of any one of claims 29-31 operably linked to control sequences recognized by a host cell transformed with the vector.
34. A host cell comprising the vector of claim 33.
35. A process of using a nucleic acid molecule encoding the WSX receptor to effect production of the WSX receptor comprising culturing the host cell of claim 34.
- 25 36. A method for enhancing proliferation or differentiation of a cell comprising the WSX receptor comprising exposing the cell to an amount of WSX ligand which is effective for enhancing proliferation or differentiation of the cell.
37. The method of claim 36 wherein the WSX receptor is the WSX receptor variant 13.2.
38. The method of claim 36 wherein the cell is a hematopoietic progenitor cell.
- 30 39. The method of claim 36 wherein the WSX ligand is OB protein.
40. The method of claim 36 wherein the WSX ligand is an anti-WSX receptor agonist antibody.
41. The method of claim 36 which enhances proliferation or differentiation of lymphoid blood cell lineages.
42. The method of claim 36 which enhances proliferation or differentiation of myeloid blood cell lineages.
- 35

43. The method of claim 36 which enhances proliferation or differentiation of erythroid blood cell lineages.
44. The method of claim 36 further comprising exposing the cell to a further cytokine.
45. The method of claim 44 wherein the further cytokine is a lineage-specific cytokine.
- 5 46. The method of claim 36 wherein the cell is present in a mammal.
47. The method of claim 46 wherein the mammal is a human.
48. The method of claim 46 wherein the mammal is suffering from, or is expected to suffer from, decreased blood cell levels.
49. The method of claim 48 wherein the decreased blood cell levels are caused by chemotherapy, radiation therapy, or bone marrow transplantation therapy.
- 10 50. A method for repopulating blood cells in a mammal comprising administering to the mammal a therapeutically effective amount of a WSX ligand.
51. The method of claim 50 wherein the blood cells are erythroid cells.
52. The method of claim 50 wherein the blood cells are myeloid cells.
- 15 53. The method of claim 50 wherein the blood cells are lymphoid cells.
54. The method of claim 50 comprising administering a further cytokine to the mammal in an amount which leads to a synergistic repopulation of the blood cells in the mammal.
55. A pharmaceutical composition comprising WSX ligand, a further cytokine, and a physiologically acceptable carrier.
- 20 56. An article of manufacture, comprising:  
a container;  
a label on the container; and  
a composition comprising an active agent contained within the container; wherein the composition is effective for repopulating blood cells in a mammal, the label on the container indicates that the composition can be used for repopulating blood cells in a mammal and the active agent in the composition is a WSX ligand.
- 25 57. The article of manufacture of claim 56 comprising a further container which holds a further cytokine.
58. An article of manufacture, comprising:  
a container;  
30 a label on the container; and  
a composition comprising an active agent contained within the container; wherein the composition is effective for decreasing body weight or fat-depot weight or decreasing food intake in an obese mammal, the label on the container indicates that the composition can be used for treating obesity in a mammal and the active agent in the composition is an agonist anti-WSX receptor antibody.

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sites: std  
length: 4102 (circular)

pleI  
hinfI  
xhoI salI  
paer7I taqI aluI  
ecoRI taqI hincII/hindII tru9I  
apoi avai accI acII mseI  
1 GAATTCCTGA GTCGACGCG GCGGTAAAG CTCCTGTGGC ATTATCCTTC AGTGGGGCTA TTGGACTGAC TTTTCTTATG CTGGGATGTG CTTAGAGGA  
CTTAAGAGCT CAGCTGCCG CCGCAATTTC GAGAGCACCG TAATAGGAAG TCACCCCGAT AACCTGACTG AAAGAATAC GACCTACAC GGAATCTCCT

rsal  
msp6I  
101 TTATGGGTGT ACTTCTCTGA AGTAAGATGA TTTGTCAAA ATTCTGTGTG GTTTGTGTAC ATTGGGAATT TATTATGTG ATACTGCGT TTAATCTGTC  
AATACCCACA TGAAGAGACT TCATTCTACT AACAGTTT TAAGACACAC CAAACAATG TAACCCCTTA ATAAATACAC TATTGACGCA AATTGAACAG

1 M I C Q K F C V V L L H W E F I Y V I T A F N L S

maeIII  
apoI  
tru9I  
mseI

201 ATATCCAATT ACTCCTTGA GATTTAAGTT GTCTTGCTATG CCACCAATT CAACCTATGA CTACTTCCTT TTGCCCTGCTG GACTCTCAA GAATACTTCA  
TATAGGTAA TGAGGACCT CTAAATTCAA CAGAAGCTAC GGTGGTTAA GTTGGTACT GATGAAGAA AACGGGACGAC CTGAGAGTTT CTTATGAAGT

26 Y P I T P W R F K L S C M P P N S T Y D Y F L L P A G L S K N T S

pleI  
hinfI  
apoI

301 AATTCGAATG GACATTATGA GACAGCTGTT GAACCTAAGT TTAATCAAG TGGTACTCAC TTTCTAACT TATCCAAAC AACCTTCCAC TGTGCTTTC  
TTAAGCTTAC CTGTAATACT CTGTCGACAA CTTGGATTCA AATTAAGTTC ACCATGACTG AAAAGATTGA ATAGGTTTG TTGAAGGTG ACAACGAAAG

59 N S N G H Y E T A V E P K F N S S G T H F S N L S K T T F H C C F R

rsal  
tru9I  
mseI  
rsal  
msp6I

401 GGACTGAGCA AGATAGAAC TGCTCCTTAT GTGCAGACAA CATTGAAGGA AAGACATTG TTTCAACAGT AAATCTTTA GTTTTCAAC AAATAGATGC  
CCTCACTCGT TCTATCTTTG ACGAGGAATA CACGCTGTT GTAACTTCTT TCTGTAAAC AAAGTTGTCA TTAAGAAAT CAAAAGTTG TTTATCTACG

93 S E Q D R N C S L C A D N I E G K T F V S T V N S L V F Q Q I D A

apoI  
sfanI

FIG. 1A

**FIG. 1B**

[illegible]

**FIG. 1C**

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nlaIII
sau3AI
mboI/ndeII(dam-)
dpmI(dam+)
dpmII(dam-)
bclI(dam-)
maeIII
1201 AAGATTGTTT CCTCAAAAGA GATTGTTTGG TGGATGAATT TAGCTGAGAA AATTCCTCAA AGCCAGTATG ATGTTGTGAG ATGTTGTGAG TGCATCATGTT AGCAAAGTTA
TTCTAACAAG GGGATTTTCT CTAAACAACC ACCTACTTAA ATCGACTCTT TTAAGGAGTT TCGGTCATAC TACAACACTC ACTAGTACAA TCGTTTCAAT
359 K I V P S K E I V W W M N L A E K I P Q S Q Y D V V S D H V S K V T
apoI . ddeI
fokI
alul
bsrI
apoI mnlI
bsrI
1301 CTTTTTTCAA TCTGAATGAA ACCAAACCTC GAGGAAAGTT TACCTATGAT GCAGTGTACT GCTGCAATGA ACATGAATGC CATCATCGCT ATGCTGAATT
GAAAAAAGTT AGACTTACTT TGGTTTGGAG CTCCTTTCAA ATGGATACCTA CGTCACATGA CGACGTTACT TGTACTTACG GTAGTAGCGA TACGACTTAA
393 F F N L N E T K P R G K F T Y D A V Y C C N E H E C H H R Y A E L
rsal fnu4HI bsmI
sfanI csp6I bbvI nlaIII
tru9I
rsal mseI
bsrI
1401 ATATGTGATT GATGTCAATA TCAATATCTC ATCTGAAACT GATGGGTACT TAACATAAAT GACTTGCAGA TGGTCAACCA GTACAATCCA GTCACCTGCG
TATACACTAA CTACAGTTAT AGTTATAGAG TACACTTTGA CTACCCATGA ATTGATTTTA CTGACGCTCT ACCAGTTGGT CATGTTAGT CAGTGAACGC
426 Y V I D V N I N I S C E T D G Y L T K M T C R W S T S T I Q S L A
rsal
csp6I
bsrI
maeIII
acil
1501 GAAAGCACTT TGCATATGAG GTATCATAGG AGCAGCCCTT ACTGTTCTGA TATTCATCT ATTCATCCCA TATCTGAGCC CAAAGATTGC TATTGCAGA
CTTTCGTGAA ACGTTAACTC CATAGTATCC TCGTCGGGAA TGACAAGACT ATAGGTAGA TAAGTAGGGT ATAGACTCGG GTTCTTAACG ATAAAGTCT
459 E S T L Q L R Y H R S S L Y C S D I P S I H P I S E P K D C Y L Q S
fokI
fnu4HI
bbvI
mnlI mnlI

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FIG. 1D



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sau3AI
mboI/ndeII[dam-]
dpoI[dam+]
dpnII[dam-]
alwI[dam-]
mami[dam-]
maeI
pleI
1601 GTGATGGTTT TTATGAATGC ATTTCCAGC CAATCTTCCT ATTATCTGGC TACCAATGT GATTAAGGT CAATCACTCT CTAGGTTTCC TTGACTCTCC
CACTACCAA AATACTTACG TAAAGGTGCG GTTGAAGGA TAATAGACCG ATGTGTACA CCTAATCCTA GTTAGTGAGA GATCCAAGTG AACTGAGAGG
493 D G F Y E C I F Q P I F L L S G Y T M W I R I N H S L G S L D S P
^begin13-2
ppu10I
nsiI/avaII
bsmI
1701 GTGATGGTTT TTATGAATGC ATTTCCAGC CAATCTTCCT ATTATCTGGC TACCAATGT GATTAAGGT CAATCACTCT CTAGGTTTCC TTGACTCTCC
CACTACCAA AATACTTACG TAAAGGTGCG GTTGAAGGA TAATAGACCG ATGTGTACA CCTAATCCTA GTTAGTGAGA GATCCAAGTG AACTGAGAGG
493 D G F Y E C I F Q P I F L L S G Y T M W I R I N H S L G S L D S P
^begin13-2
nlaIII
nspI
nspHI
afIII
tfII
hinfi
hphI
fokI
bsrI
1701 ACCAATCATGT GTCCTTCCTG ATTCTGTGGT GAAGCCACTG CCTCCATCCA GTGTGAAGC AGAATTAATCT ATAAACATTTG GATTATTGAA AATATCTTGG
TGGTTGTACA CAGGAAGGAC TAAGACACCA CTTCGGTGAC GGAGGTAGGT CACTTTTCG TCTTTAATGA TATTTGTAAC CTAATAACTT TTATAGAACC
526 P T C V L P D S V V K P L P S S V K A E I T I N I G L L K I S W
tfII
hinfi
xcmI
bsrI
1801 GAAAGCCAG TCTTTCAGA GAATAACCTT CAATTCAGG TTCGCTATGG TTAAGTGGA AAAGAAGTAC AATGAAGAT GTATGAGGTT TATGATGCAA
CTTTCCGTC AGAAGGTCT CTATTGGAA GTTAAGTCTT AAGCATACC AAATCACCT TTTCTTCATG TTACCTCTTA CATACTCCAA ATACTACGTT
559 E K P V F P E N N L Q F Q I R Y G L S G K E V Q W K M Y E V Y D A K
bsmAI bsrI
1901 AATCAAATC TGTCACTCTC CCAGTTCAG ACTTGTGTGC AGTCTATGCT GTTCAGGTGC GCTGTAAAG GCTAGATGGA CTGGGATATT GGAGTAATTG
TTAGTTTAG ACAGTCAGAG GGTCAGGTC TGAACACACG TCAGATACGA CAAGTCCACG CGACATCTC CGATCTACCT GACCTATAA CCTCATTTAC
593 S K S V S L P V P D L C A V Y A V Q V R C K R L D G L G Y W S N W
sau96I
avaII
asuI
ppuMI
ecoNI
eco0109I/draII
bslI
mnlI
apoI
nlaIII
2001 GAGCAATCCA GCCTACACAG TTGTCATGGA TATAAAGTT CCTATGAGAG GACCTGAATT TTGAGAATA ATTAATGGAG ATACTATGAA AAAGGAGAAA
CTCGTTAGGT CGGATGTGTC AACAGTACCT ATATTTCAA GGATACCTCTC CTGGACTTAA AACCTCTTAT TAATTACCTC TATGATACTT TTTCTCTTT
626 S N P A Y T V V M D I K V P M R G P E F W R I I N G D T M K E K
aseI/asnI/vspI

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FIG. 1E

**FIG. 1F**

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sau3AI
mboI/ndeII{dam-}
dpmI{dam+}
dpmII{dam-}
bclI{dam-}
nlaIII
2501 TTAAGAAGTA TTATATCCAT GATCATTTTA TCCCATTTGA GAATACCCAG TTCAGTCTTT ACCCAATATT TATGGAAGGA GTGGGAAGAC CAAAGATAAT
793 K K Y Y I H D H F I P I E K Y Q F S L Y P I F M E G V G K P K I I
truuI
mseI
bsrI
rsal
csp6I
sspI
aseI/asnI/vspI
2601 TAATAGTTTC ACTCAAGATG ATATTGAAAA ACACCAGAGT GATGCAGGTT TATATGTAAT TGTGCCAGTA ATTATTTCCCT CTTCCATCTT ATTGCTTGA
826 N S F T Q D D I E K H Q S D A G L Y V I V P V I I S S S I L L G
bspMI
sfaNI
bspI
earI/ksp632I
mboII
2701 ACATTATTAA TATACACCA AAGAATGAAA AAGCTATTTT GGAAGATGT TCGAACCCC AAGAATTGTT CCTGGGCACA AGGACTTAA TTTGAGAAGC
TGTAATAATT ATAGTGTGGT TTCTTACTTT TTCGATAAAA CCTTCTACA AGCTTGGGG TTCTTAACAA GGACCCGTGT TCCTGAATTA AAAGTCTTCG
859 T L L I S H Q R M K K L F W E D V P N P K N C S W A Q G L N F Q K P
truuI
mseI
aseI/asnI/vspI
aluI
asp700
xmnI
mboII
bsrI
bsaJI
truuI
mseI
apyl{dcm+}
bsp1286
bmyI
scrFI
mvaI
ecorII
dsav
bstNI
bsaJI
truuI
mseI
nlaIII
nspI
earI/ksp632I
nspHI
sau96I
afIII
avaII
eam1105I
mnlI
ecorV
801 CAGAACGTT TGAGCATCTT TTTATCAAGC ATACAGCATC AGTGACATGT GGTCTCTCTC TTTTGGAGCC TGAACAAT TCAAGAGATA TCAGTGTGA
GTCTTTGCAA ACTCGTAGAA AATAGTTCG TATGTCGTAG TACTGTACA CCAGGAGAAG AAAACCTCGG ACTTGTAA AGTCTTCTAT AGTCACAACT
893 E T F E H L F I K H T A S V T C G P L L L E P E T I S E D I S V D

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FIG. 1G

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sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
bstXI/xhoII
bsrI
maeIII
2901 TACATCATGG AAAATAAAG ATGAGATGAT GCCACAAC TACTTCAAC AACAGATCTT GAAAAGGGTT CTGTTTGTAT TAGTGACCAG
ATGTAATACC TTTTATTTC TACTCTACTA CGGTGTGTTGA CACCAGAGAG ATGAAGTTG TTGTCTAGAA CTTTCCCAA GCAAAACATA ATCACTGGTC
926 T S W K N K D E M M P T T V V S L L S T T D L E K G S V C I S D Q

bsmAI
bsaI
sfaNI
2901 TACATCATGG AAAATAAAG ATGAGATGAT GCCACAAC TACTTCAAC AACAGATCTT GAAAAGGGTT CTGTTTGTAT TAGTGACCAG
ATGTAATACC TTTTATTTC TACTCTACTA CGGTGTGTTGA CACCAGAGAG ATGAAGTTG TTGTCTAGAA CTTTCCCAA GCAAAACATA ATCACTGGTC
926 T S W K N K D E M M P T T V V S L L S T T D L E K G S V C I S D Q

tru9I
mseI
3001 TTCAACAGTG TTAACCTTTC TGAGGCTGAG GGTACTGAGG TAACCTATGA GGACGAAAGC CAGAGACAAC CCTTGTGTTAA ATACGCCACG CTGATCAGCA
AAGTGTGTCAC AATTGAAGAG ACTCCGACTC CCATGACTCC ATTGATACT CCTGCTTCG GTCTCTGTTG GGAACAATT TATCGGTGC GACTAGTGGT
959 F N S V N F S E A E G T E V T Y E D E S Q R Q P F V K Y A T L I S N

draIII
hphI
hpaI
hincII/hindII mnlI mnlI csp6I bsteII mnlI
3001 TTCAACAGTG TTAACCTTTC TGAGGCTGAG GGTACTGAGG TAACCTATGA GGACGAAAGC CAGAGACAAC CCTTGTGTTAA ATACGCCACG CTGATCAGCA
AAGTGTGTCAC AATTGAAGAG ACTCCGACTC CCATGACTCC ATTGATACT CCTGCTTCG GTCTCTGTTG GGAACAATT TATCGGTGC GACTAGTGGT
959 F N S V N F S E A E G T E V T Y E D E S Q R Q P F V K Y A T L I S N

bsrI mboII
3101 ACTCTAAACC AAGTGAACT GGTGAGAAC AAGGGCTTAT AAATAGTTCA GTCACCAAGT GCTTCTCTAG CAAAATTTCT CCGTTGAAGG ATTCTTTCTC
TGAGATTGG TTCACITGGA CCACITCTTG TTCCCGAATA TTTATCAAGT CAGTGGTTCA CGAAGAGATC GTTTTAAAG GGAACACTCC TAAGAAAGAG
993 S K P S E T G E E Q G L I N S S V T K C F S S K N S P L K D S F S

scrFI
mvaI
ecorII
dsav
bstNI
apyI[dcn+]
sau96I
haeIII/palI
asul
nlaIII
aluI
3201 TAATAGCTCA TGGGAGATAG AGGCCAGGC ATTTTATATA TTATCAGATC AGCATCCCAA CATAATTTC CCACACTCA CATTCTCAGA AGGATTGGAT
ATTATCGAGT ACCCTCTATC TCCGGGTCCG TAAAAATAT AATAGTCTAG TCCTAGGGTT GTATTAAAGT GGTGTGAGT GTAACAGTCT TCCTAACCTA
1026 N S S W E I E A Q A F F I L S D Q H P N I I S P H L T F S E G L D

sau3AI
mboI/ndeII[dam-]
dpnI[dam+]
dpnII[dam-]
bstXI/xhoII
bsrI
maeIII
2901 TACATCATGG AAAATAAAG ATGAGATGAT GCCACAAC TACTTCAAC AACAGATCTT GAAAAGGGTT CTGTTTGTAT TAGTGACCAG
ATGTAATACC TTTTATTTC TACTCTACTA CGGTGTGTTGA CACCAGAGAG ATGAAGTTG TTGTCTAGAA CTTTCCCAA GCAAAACATA ATCACTGGTC
926 T S W K N K D E M M P T T V V S L L S T T D L E K G S V C I S D Q

bsmAI
bsaI
sfaNI
2901 TACATCATGG AAAATAAAG ATGAGATGAT GCCACAAC TACTTCAAC AACAGATCTT GAAAAGGGTT CTGTTTGTAT TAGTGACCAG
ATGTAATACC TTTTATTTC TACTCTACTA CGGTGTGTTGA CACCAGAGAG ATGAAGTTG TTGTCTAGAA CTTTCCCAA GCAAAACATA ATCACTGGTC
926 T S W K N K D E M M P T T V V S L L S T T D L E K G S V C I S D Q

tru9I
mseI
3001 TTCAACAGTG TTAACCTTTC TGAGGCTGAG GGTACTGAGG TAACCTATGA GGACGAAAGC CAGAGACAAC CCTTGTGTTAA ATACGCCACG CTGATCAGCA
AAGTGTGTCAC AATTGAAGAG ACTCCGACTC CCATGACTCC ATTGATACT CCTGCTTCG GTCTCTGTTG GGAACAATT TATCGGTGC GACTAGTGGT
959 F N S V N F S E A E G T E V T Y E D E S Q R Q P F V K Y A T L I S N

draIII
hphI
hpaI
hincII/hindII mnlI mnlI csp6I bsteII mnlI
3001 TTCAACAGTG TTAACCTTTC TGAGGCTGAG GGTACTGAGG TAACCTATGA GGACGAAAGC CAGAGACAAC CCTTGTGTTAA ATACGCCACG CTGATCAGCA
AAGTGTGTCAC AATTGAAGAG ACTCCGACTC CCATGACTCC ATTGATACT CCTGCTTCG GTCTCTGTTG GGAACAATT TATCGGTGC GACTAGTGGT
959 F N S V N F S E A E G T E V T Y E D E S Q R Q P F V K Y A T L I S N

bsrI mboII
3101 ACTCTAAACC AAGTGAACT GGTGAGAAC AAGGGCTTAT AAATAGTTCA GTCACCAAGT GCTTCTCTAG CAAAATTTCT CCGTTGAAGG ATTCTTTCTC
TGAGATTGG TTCACITGGA CCACITCTTG TTCCCGAATA TTTATCAAGT CAGTGGTTCA CGAAGAGATC GTTTTAAAG GGAACACTCC TAAGAAAGAG
993 S K P S E T G E E Q G L I N S S V T K C F S S K N S P L K D S F S

scrFI
mvaI
ecorII
dsav
bstNI
apyI[dcn+]
sau96I
haeIII/palI
asul
nlaIII
aluI
3201 TAATAGCTCA TGGGAGATAG AGGCCAGGC ATTTTATATA TTATCAGATC AGCATCCCAA CATAATTTC CCACACTCA CATTCTCAGA AGGATTGGAT
ATTATCGAGT ACCCTCTATC TCCGGGTCCG TAAAAATAT AATAGTCTAG TCCTAGGGTT GTATTAAAGT GGTGTGAGT GTAACAGTCT TCCTAACCTA
1026 N S S W E I E A Q A F F I L S D Q H P N I I S P H L T F S E G L D

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FIG. 1H

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mnII
hphI
maeIII
bstEII
3301 GAACCTTTTGA AATTGGAGGG AAATTTCCTT GAAGAAATA ATGATAAAA GTCTATCTAT TATTTAGGGG TCACCTCAAT CAAAAAGAGA GAGAGTGGTG
CTTGAAACTT TTAACCTCC TTTAAGGGA CTTCTTTTAT TACTATTTT CAGATAGATA ATAAATCCCC AGTGGAGTTA GTTTTCTCT CTCTCACCAC
1059 E L L K L E G N F P E E N N D K K S I Y Y L G V T S I K K R E S G V

mnII
apoI
mboII
eco57I
3401 TGCTTTTGAC TGACAAGTCA AGGTATCGT GCCCATTCCT AGCCCTCTGT TTATTCACGG ACATCAGAGT TCTCCAGGAC AGTTGCTCAC ACTTTGTAGA
ACGAAACTG ACTGTTTCAGT TCCCATAGCA CGGTAAGGG TCGGGGGACA AATAAGTGCC TGTAGTCTCA AGAGGTCCTG TCAACGAGTG TGAACATCT
1093 L L T D K S R V S C P F P A P C L F T D I R V L Q D S C S H F V E

drdI
bsp1286
bmyI
3401 TGCTTTTGAC TGACAAGTCA AGGTATCGT GCCCATTCCT AGCCCTCTGT TTATTCACGG ACATCAGAGT TCTCCAGGAC AGTTGCTCAC ACTTTGTAGA
ACGAAACTG ACTGTTTCAGT TCCCATAGCA CGGTAAGGG TCGGGGGACA AATAAGTGCC TGTAGTCTCA AGAGGTCCTG TCAACGAGTG TGAACATCT
1093 L L T D K S R V S C P F P A P C L F T D I R V L Q D S C S H F V E

maeIII
nlaIII
nspI
sfalI
nspHI
mnII
dclI
3501 AAATAATATC AACTTAGGAA CTTCTAGTAA GRAGACTTTT GCATCTTACA TGCTCAATT CCAAACTTGT TCTACTCAGA CTCATAAGAT CATGGAAGAC
TTTATTATAG TTGAATCCTT GAAGATCATT CTTCTGAAA CGTAGAATCT ACGGAGTTAA GGTTGAACA AGATGAGTCT GAGTATTCTA GTACCTTTTG
1126 N N I N L G T S S K K T F A S Y M P Q F Q T C S T Q T H K I M E N

maeIII
eco57I
3601 AAGATGTGTG ACCTTAAGTGT GTAATTTCAC TGAAGAAACC TTCAGATTGT TGTATAATG GGTAAATATA AGTGAATAG ATTATAGTTG TGGTGGGAG
TTCTACACAC TGGATTGACA CATTAAAGTG ACTTCTTTGG AAGTCTAAAC ACAATATTAC CCATTATATT TCACATTATC TAATATCAAC ACCACCCTC
1159 K M C D L T V

pleI
hinfI
apoI
3701 AGAGAAAGA AACCAAGATC AAATTGAAA ATAATTGTTT CAAATGAATG TTGCTGTGTT GTTCTCTCTT AGTAACATAG ACAAATAATT TGAGAAAGCC
TCTCTTTTCT TTGGTCTCAG TTAAACTTT TATTAACAAG GTTTACTTAC AACAGACAAA CAAGAGAGAA TCATTGTATC TGTTTTAA ACTCTTTCCG

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FIG. 1I

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sau96I
nlaiV
avaII
asulI
ppuHI
ecoo109I/draII
alulI
rmaI
maeI
3801 TTCATAAGCC TACCAATGTA GACACGCTCT TCTATTTTAT TCCCAAGCTC TAGTGGGAAG GTCCCTTGTT TCCAGCTAGA AATAAGCCCC ACAGACACCA
AAGTATTCCG ATGGTTACAT CTGTGCGAGA AGATAAATA AGGTTTCGAG ATCACCCCTC CAGGGAACAA AGTCCGATCT TTATTGGGT TGTCTGTGCT
mboII
earI/ksp632I
sapi
accI
3901 TCTTTGTGA GATGTAATTG TTTTTCAGA GGGCGGTGTG TTTTACCTCA AGTTTTGTT TGTACCAAC ACACACACAC ACACACATTC TTAACACATG
AGAAACACT CTACATTAAC AAAAAGCTT CCCGCACAC AAAATGGAGT TCAAAACAA AACATGCTG TGTGTGTG TGTTGTAG AATTGTGTAC
nspi
nspHI
tru9I nlaiII
mseI aflIII
4001 TCCTTGTGTG TTTTGAGAGT ATATTATGTA TTTATATTT GTGCTATCAG ACTGTAGGAT TTGAAGTAGG ACTTTCCTAA ATGTTTAAGA TAAACAGAT
AGGAACACAC AAACCTCTCA TATAATACAT AAATATAAAA CACGATGTC TGACATCCTA AACTCATCC TGAAGGAT TACAAATTCT ATTTGCTTA
taqI
4101 TC
AG
length: 4102

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FIG. 1J

wsxfull.6.4.variant	1	M I C Q K F C V V L L H W E F I Y V I T A F N L S Y P I T P W R F K L S C M P P N S T Y D Y F L L P
wsxfull.12.1.variant	1	M I C Q K F C V V L L H W E F I Y V I T A F N L S Y P I T P W R F K L S C M P P N S T Y D Y F L L P
wsxfull.13.2.variant	1	M I C Q K F C V V L L H W E F I Y V I T A F N L S Y P I T P W R F K L S C M P P N S T Y D Y F L L P
wsxfull.6.4.variant	51	A G L S K N T S N S N G H Y E T A V E P K F N S S G T H F S N L S K T T F H C C F R S E Q D R N C S
wsxfull.12.1.variant	51	A G L S K N T S N S N G H Y E T A V E P K F N S S G T H F S N L S K T T F H C C F R S E Q D R N C S
wsxfull.13.2.variant	51	A G L S K N T S N S N G H Y E T A V E P K F N S S G T H F S N L S K T T F H C C F R S E Q D R N C S
wsxfull.6.4.variant	101	L C A D N I E G K T F V S T V N S L V F Q Q I D A N W N I Q C W L K G D L K L F I C Y V E S L F K N
wsxfull.12.1.variant	101	L C A D N I E G K T F V S T V N S L V F Q Q I D A N W N I Q C W L K G D L K L F I C Y V E S L F K N
wsxfull.13.2.variant	101	L C A D N I E G K T F V S T V N S L V F Q Q I D A N W N I Q C W L K G D L K L F I C Y V E S L F K N
wsxfull.6.4.variant	151	L F R N Y N Y K V H L L Y V L P E V L E D S P L V P Q K G S F Q M V H C N C S V H E C C E C L V P V
wsxfull.12.1.variant	151	L F R N Y N Y K V H L L Y V L P E V L E D S P L V P Q K G S F Q M V H C N C S V H E C C E C L V P V
wsxfull.13.2.variant	151	L F R N Y N Y K V H L L Y V L P E V L E D S P L V P Q K G S F Q M V H C N C S V H E C C E C L V P V
wsxfull.6.4.variant	201	P T A K L N D T L L M C L K I T S G G V I F O S P L M S V Q P I N M V K P D P P L G L H M E I T D D
wsxfull.12.1.variant	201	P T A K L N D T L L M C L K I T S G G V I F O S P L M S V Q P I N M V K P D P P L G L H M E I T D D
wsxfull.13.2.variant	201	P T A K L N D T L L M C L K I T S G G V I F O S P L M S V Q P I N M V K P D P P L G L H M E I T D D
wsxfull.6.4.variant	251	G N L K I S W S S P P L V P F P L Q Y Q V K Y S E N S T T V I R E A D K I V S A T S L L V D S I L P
wsxfull.12.1.variant	251	G N L K I S W S S P P L V P F P L Q Y Q V K Y S E N S T T V I R E A D K I V S A T S L L V D S I L P
wsxfull.13.2.variant	251	G N L K I S W S S P P L V P F P L Q Y Q V K Y S E N S T T V I R E A D K I V S A T S L L V D S I L P

FIG. 2A

wsxfull.6.4.variant	301	GSSYE VQVRGKRLDGGGIWSDWSTPRVFTTQDV IYFPKILTSVGSNVSF
wsxfull.12.1.variant	301	GSSYE VQVRGKRLDGGGIWSDWSTPRVFTTQDV IYFPKILTSVGSNVSF
wsxfull.13.2.variant	301	GSSYE VQVRGKRLDGGGIWSDWSTPRVFTTQDV IYFPKILTSVGSNVSF
wsxfull.6.4.variant	351	HC IYKKENK I VPSKE I V WWMNL AEK I PQSOYDV VSDHVS KVTFFNLNETK
wsxfull.12.1.variant	351	HC IYKKENK I VPSKE I V WWMNL AEK I PQSOYDV VSDHVS KVTFFNLNETK
wsxfull.13.2.variant	351	HC IYKKENK I VPSKE I V WWMNL AEK I PQSOYDV VSDHVS KVTFFNLNETK
wsxfull.6.4.variant	401	PRGKFTYDAVYCCNEHECHHRYAEIYVIDVNI NISCETDGYLTKMTCRWS
wsxfull.12.1.variant	401	PRGKFTYDAVYCCNEHECHHRYAEIYVIDVNI NISCETDGYLTKMTCRWS
wsxfull.13.2.variant	401	PRGKFTYDAVYCCNEHECHHRYAEIYVIDVNI NISCETDGYLTKMTCRWS
wsxfull.6.4.variant	451	TSTIQSLAESTLQLRYHRSSLYCSDIPSIHP ISEPKDCYLQSDGFYECIF
wsxfull.12.1.variant	451	TSTIQSLAESTLQLRYHRSSLYCSDIPSIHP ISEPKDCYLQSDGFYECIF
wsxfull.13.2.variant	451	TSTIQSLAESTLQLRYHRSSLYCSDIPSIHP ISEPKDCYLQSDGFYECIF
wsxfull.6.4.variant	501	OPIFLLSGYTMWIRINHSLSLSLDSPTTCVLPDSVVKPLPPSSVKAETIN
wsxfull.12.1.variant	501	OPIFLLSGYTMWIRINHSLSLSLDSPTTCVLPDSVVKPLPPSSVKAETIN
wsxfull.13.2.variant	501	OPIFLLSGYTMWIRINHSLSLSLDSPTTCVLPDSVVKPLPPSSVKAETIN
wsxfull.6.4.variant	551	IGLLKISWEKPVFPENNLOFOIRYGLSGKEVQWKMYEYDAKSKSVSLPV
wsxfull.12.1.variant	551	IGLLKISWEKPVFPENNLOFOIRYGLSGKEVQWKMYEYDAKSKSVSLPV
wsxfull.13.2.variant	551	IGLLKISWEKPVFPENNLOFOIRYGLSGKEVQWKMYEYDAKSKSVSLPV

FIG. 2B



wsxfull.6.4.variant	601	P D L C A V Y A V Q V R C K R L D G L G Y W S N W S N P A Y T V V M D I K V P M R G P E F W R I I N
wsxfull.12.1.variant	601	P D L C A V Y A V Q V R C K R L D G L G Y W S N W S N P A Y T V V M D I K V P M R G P E F W R I I N
wsxfull.13.2.variant	601	P D L C A V Y A V Q V R C K R L D G L G Y W S N W S N P A Y T V V M D I K V P M R G P E F W R I I N
wsxfull.6.4.variant	651	G D T M K K E K N V T L L W K P L M K N D S L C S V Q R Y V I N H H T S C N G T W S E D V G N H T K
wsxfull.12.1.variant	651	G D T M K K E K N V T L L W K P L M K N D S L C S V Q R Y V I N H H T S C N G T W S E D V G N H T K
wsxfull.13.2.variant	651	G D T M K K E K N V T L L W K P L M K N D S L C S V Q R Y V I N H H T S C N G T W S E D V G N H T K
wsxfull.6.4.variant	701	F T F L W T E O A H T V T V L A I N S I G A S V A N F N L T F S W P M S K V N I V Q S L S A Y P L N
wsxfull.12.1.variant	701	F T F L W T E O A H T V T V L A I N S I G A S V A N F N L T F S W P M S K V N I V Q S L S A Y P L N
wsxfull.13.2.variant	701	F T F L W T E O A H T V T V L A I N S I G A S V A N F N L T F S W P M S K V N I V Q S L S A Y P L N
wsxfull.6.4.variant	751	S S C V I V S W I L S P S D Y K L M Y F I I E W K N L N E D G E I K W L R I S S S V K K Y Y I H D H
wsxfull.12.1.variant	751	S S C V I V S W I L S P S D Y K L M Y F I I E W K N L N E D G E I K W L R I S S S V K K Y Y I H D H
wsxfull.13.2.variant	751	S S C V I V S W I L S P S D Y K L M Y F I I E W K N L N E D G E I K W L R I S S S V K K Y Y I H D H
wsxfull.6.4.variant	801	F I P I E K Y Q F S L Y P I F M E G V G K P K I I N S F T O D D I E K H O S D A G L Y V I V P V I I
wsxfull.12.1.variant	801	F I P I E K Y Q F S L Y P I F M E G V G K P K I I N S F T O D D I E K H O S D A G L Y V I V P V I I
wsxfull.13.2.variant	801	F I P I E K Y Q F S L Y P I F M E G V G K P K I I N S F T O D D I E K H O S D A G L Y V I V P V I I
wsxfull.6.4.variant	851	S S I L L L G T L L I S H Q R M K K L F W E D V P N P K N C S W A Q G L N F O K
wsxfull.12.1.variant	851	S S I L L L G T L L I S H Q R M K K L F W E D V P N P K N C S W A Q G L N F O K
wsxfull.13.2.variant	851	S S I L L L G T L L I S H Q R M K K L F W E D V P N P K N C S W A Q G L N F O K

Trans

Box 1

membrane Domain

FIG. 2C



wsxfull.6.4.variant	1	G A A T T C C G G G T T A A A G C T C T C G T G G C A T T A T C C T T C A G T G G G G C T A T T G G
wsxfull.6.4.variant	51	A C T G A C T T T T C T T A T G C T G G G A T G T G C C T T A G A G G A T T A T G G A T T T G C C A
wsxfull.12.1.variant	1	.....G A A T T C T C G A G T C
wsxfull.13.2.variant	1	.....G A A T T C T C G A G T C
wsxfull.6.4.variant	101	G T T C A C C C T G A C C A T C T T G A A A A T A A G T T A T C T C T G A T C T C T G T C T G T A T
wsxfull.12.1.variant	14	G A C G G C G G G C G T T A A A G C T C T C G T G G C A T T A T C C T T C A G T G G G G C T A T T G
wsxfull.13.2.variant	14	G A C G G C G G G C G T T A A A G C T C T C G T G G C A T T A T C C T T C A G T G G G G C T A T T G
wsxfull.6.4.variant	151	G T T A C T T C T C T C C C T C A C C A A T G G A G A C A A T G T G G G C A A A G T G T A C T
wsxfull.12.1.variant	64	G A C T G A C T T T T C T T A T G C T G G G A T G T G C C T T A G A G G A T T A T G G G T G T A C T
wsxfull.13.2.variant	64	G A C T G A C T T T T C T T A T G C T G G G A T G T G C C T T A G A G G A T T A T G G G T G T A C T
wsxfull.6.4.variant	201	T C T C T G A A G T A A G A T G A T T T G T C A A A A A T T C T G T G T G G T T T T G T T A C A T T
wsxfull.12.1.variant	114	T C T C T G A A G T A A G A T G A T T T G T C A A A A A T T C T G T G T G G T T T T G T T A C A T T
wsxfull.13.2.variant	114	T C T C T G A A G T A A G A T G A T T T G T C A A A A A T T C T G T G T G G T T T T G T T A C A T T
wsxfull.6.4.variant	251	G G G A T T T A T T T A T G T G A T A A C T G C G T T T A A C T T G T C A T A T C C A A T T A C T
wsxfull.12.1.variant	164	G G G A T T T A T T T A T G T G A T A A C T G C G T T T A A C T T G T C A T A T C C A A T T A C T
wsxfull.13.2.variant	164	G G G A T T T A T T T A T G T G A T A A C T G C G T T T A A C T T G T C A T A T C C A A T T A C T
wsxfull.6.4.variant	301	C C T T G G A G A T T T A A G T T G T C T T G C A T G C C A C C A A A T T C A A C C T A T G A C T A
wsxfull.12.1.variant	214	C C T T G G A G A T T T A A G T T G T C T T G C A T G C C A C C A A A T T C A A C C T A T G A C T A
wsxfull.13.2.variant	214	C C T T G G A G A T T T A A G T T G T C T T G C A T G C C A C C A A A T T C A A C C T A T G A C T A

FIG. 3A

wsxfull.6.4.variant	351	C T T C C T T T T G C C T G C T G G A C T C T C A A G A A T A C T T C A A A T T C G A A T G G A C
wsxfull.12.1.variant	264	C T T C C T T T T G C C T G C T G G A C T C T C A A G A A T A C T T C A A A T T C G A A T G G A C
wsxfull.13.2.variant	264	C T T C C T T T T G C C T G C T G G A C T C T C A A G A A T A C T T C A A A T T C G A A T G G A C
wsxfull.6.4.variant	401	A T T A T G A G A C A G C T G T T G A A C C T A A G T T T A A T T C A A G T G G T A C T C A C T T T
wsxfull.12.1.variant	314	A T T A T G A G A C A G C T G T T G A A C C T A A G T T T A A T T C A A G T G G T A C T C A C T T T
wsxfull.13.2.variant	314	A T T A T G A G A C A G C T G T T G A A C C T A A G T T T A A T T C A A G T G G T A C T C A C T T T
wsxfull.6.4.variant	451	T C T A A C T T A T C C A A A C A A C A C T T T C C A C T G T T G C T T T C G G A G T G A G C A A G A
wsxfull.12.1.variant	364	T C T A A C T T A T C C A A A C A A C A C T T T C C A C T G T T G C T T T C G G A G T G A G C A A G A
wsxfull.13.2.variant	364	T C T A A C T T A T C C A A A C A A C A C T T T C C A C T G T T G C T T T C G G A G T G A G C A A G A
wsxfull.6.4.variant	501	T A G A A A C T G C T C C T T A T G T G C A G A C A A C A T T G A A G G A A A G A C A T T T G T T T
wsxfull.12.1.variant	414	T A G A A A C T G C T C C T T A T G T G C A G A C A A C A T T G A A G G A A A G A C A T T T G T T T
wsxfull.13.2.variant	414	T A G A A A C T G C T C C T T A T G T G C A G A C A A C A T T G A A G G A A A G A C A T T T G T T T
wsxfull.6.4.variant	551	C N A C A G T A A A T T C T T T A G T T T T C A A C A A A T A G A T G C A A A C T G G A A C A T A
wsxfull.12.1.variant	464	C A A C A G T A A A T T C T T T A G T T T T C A A C A A A T A G A T G C A A A C T G G A A C A T A
wsxfull.13.2.variant	464	C A A C A G T A A A T T C T T T A G T T T T C A A C A A A T A G A T G C A A A C T G G A A C A T A
wsxfull.6.4.variant	601	C A G T G C T G G C T A A A A G G A G A C T T A A A A T T A T T C A T C T G T T A T G T G G A G T C
wsxfull.12.1.variant	514	C A G T G C T G G C T A A A A G G A G A C T T A A A A T T A T T C A T C T G T T A T G T G G A G T C
wsxfull.13.2.variant	514	C A G T G C T G G C T A A A A G G A G A C T T A A A A T T A T T C A T C T G T T A T G T G G A G T C

FIG. 3B

wsxfull.6.4.variant	651	A T T A T T T A A G A A T C T A T T C A G G A A T T A A C T A T A A G G T C C A T C T T T A T
wsxfull.12.1.variant	664	A T T A T T T A A G A A T C T A T T C A G G A A T T A A C T A T A A G G T C C A T C T T T A T
wsxfull.13.2.variant	664	A T T A T T T A A G A A T C T A T T C A G G A A T T A A C T A T A A G G T C C A T C T T T A T
wsxfull.6.4.variant	701	A T G T T C T G C C T G A A G T G T T A G A A G A T T C A C C T C T G G T T C C C C A A A A G G C
wsxfull.12.1.variant	664	A T G T T C T G C C T G A A G T G T T A G A A G A T T C A C C T C T G G T T C C C C A A A A G G C
wsxfull.13.2.variant	664	A T G T T C T G C C T G A A G T G T T A G A A G A T T C A C C T C T G G T T C C C C A A A A G G C
wsxfull.6.4.variant	751	A G T T T T C A G A T G G T T C A C T G C A A T T G C A G T G T T C A T G A A T G T G A A T G
wsxfull.12.1.variant	664	A G T T T T C A G A T G G T T C A C T G C A A T T G C A G T G T T C A T G A A T G T G A A T G
wsxfull.13.2.variant	664	A G T T T T C A G A T G G T T C A C T G C A A T T G C A G T G T T C A T G A A T G T G A A T G
wsxfull.6.4.variant	801	T C T T G T G C C T G T G C C A A C A G C C A A A C T C A A C G A C A C T C T C C T T A T G T G T T
wsxfull.12.1.variant	714	T C T T G T G C C T G T G C C A A C A G C C A A A C T C A A C G A C A C T C T C C T T A T G T G T T
wsxfull.13.2.variant	714	T C T T G T G C C T G T G C C A A C A G C C A A A C T C A A C G A C A C T C T C C T T A T G T G T T
wsxfull.6.4.variant	851	T G A A A T C A C A T C T G G T G G A G T A A T T T C C A G T C A C C T C T A A T G T C A G T T
wsxfull.12.1.variant	764	T G A A A T C A C A T C T G G T G G A G T A A T T T C C A G T C A C C T C T A A T G T C A G T T
wsxfull.13.2.variant	764	T G A A A T C A C A T C T G G T G G A G T A A T T T C C A G T C A C C T C T A A T G T C A G T T
wsxfull.6.4.variant	901	C A G C C C A T A A A T A T G G T G A A G C C T G A T C C A C C A T T A G G T T T G C A T A T G G A
wsxfull.12.1.variant	814	C A G C C C A T A A A T A T G G T G A A G C C T G A T C C A C C A T T A G G T T T G C A T A T G G A
wsxfull.13.2.variant	814	C A G C C C A T A A A T A T G G T G A A G C C T G A T C C A C C A T T A G G T T T G C A T A T G G A

FIG. 3C

wsxfull.6.4.variant	951	AATCACAGATGATGGTAATTAAAGATTTCCTTGGTCCAGGCCACCCATTGG
wsxfull.12.1.variant	864	AATCACAGATGATGGTAATTAAAGATTTCCTTGGTCCAGGCCACCCATTGG
wsxfull.13.2.variant	864	AATCACAGATGATGGTAATTAAAGATTTCCTTGGTCCAGGCCACCCATTGG
wsxfull.6.4.variant	1001	TACCAATTTCCACTTCAATATCAAGTGAAATATTCAGAGAAATTCACAAACA
wsxfull.12.1.variant	914	TACCAATTTCCACTTCAATATCAAGTGAAATATTCAGAGAAATTCACAAACA
wsxfull.13.2.variant	914	TACCAATTTCCACTTCAATATCAAGTGAAATATTCAGAGAAATTCACAAACA
wsxfull.6.4.variant	1051	GTTATCAGAGAAGCTGACAAAGATTGTCTCAGCTACATCCCTGCTAGTAGA
wsxfull.12.1.variant	964	GTTATCAGAGAAGCTGACAAAGATTGTCTCAGCTACATCCCTGCTAGTAGA
wsxfull.13.2.variant	964	GTTATCAGAGAAGCTGACAAAGATTGTCTCAGCTACATCCCTGCTAGTAGA
wsxfull.6.4.variant	1101	CAGTATACTTCCCTGGGTCTTCGGTATGAGGTTCAAGGTGAGGGGCAAGAGAC
wsxfull.12.1.variant	1014	CAGTATACTTCCCTGGGTCTTCGGTATGAGGTTCAAGGTGAGGGGCAAGAGAC
wsxfull.13.2.variant	1014	CAGTATACTTCCCTGGGTCTTCGGTATGAGGTTCAAGGTGAGGGGCAAGAGAC
wsxfull.6.4.variant	1151	TGGATGGCCCAAGGAATCTGGAGTGACTGGAGTACTCCTCGTGTCTTTACC
wsxfull.12.1.variant	1064	TGGATGGCCCAAGGAATCTGGAGTGACTGGAGTACTCCTCGTGTCTTTACC
wsxfull.13.2.variant	1064	TGGATGGCCCAAGGAATCTGGAGTGACTGGAGTACTCCTCGTGTCTTTACC
wsxfull.6.4.variant	1201	ACACAAGATGTCAATATACTTTCACCTAAATAATTCAGACAAGTGTGGGTC
wsxfull.12.1.variant	1114	ACACAAGATGTCAATATACTTTCACCTAAATAATTCAGACAAGTGTGGGTC
wsxfull.13.2.variant	1114	ACACAAGATGTCAATATACTTTCACCTAAATAATTCAGACAAGTGTGGGTC

FIG. 3D

wsxfull.6.4.variant	1251	T A A T G T T T C T T T T C A C T G C A T C T A T A G A A G G A A A C A A G A T T G T T C C C T
wsxfull.12.1.variant	1164	T A A T G T T T C T T T T C A C T G C A T C T A T A G A A G G A A A C A A G A T T G T T C C C T
wsxfull.13.2.variant	1164	T A A T G T T T C T T T T C A C T G C A T C T A T A G A A G G A A A C A A G A T T G T T C C C T
wsxfull.6.4.variant	1301	C A A A G A G A T T G T T T G G T G G A T G A A T T A G C T G A G A A A A T T C C T C A A A G C
wsxfull.12.1.variant	1214	C A A A G A G A T T G T T T G G T G G A T G A A T T A G C T G A G A A A A T T C C T C A A A G C
wsxfull.13.2.variant	1214	C A A A G A G A T T G T T T G G T G G A T G A A T T A G C T G A G A A A A T T C C T C A A A G C
wsxfull.6.4.variant	1351	C A G T A T G A T G T T G T G A G T G A T C A T G T T A G C A A A G T T A C T T T T T C A A T C T
wsxfull.12.1.variant	1264	C A G T A T G A T G T T G T G A G T G A T C A T G T T A G C A A A G T T A C T T T T T C A A T C T
wsxfull.13.2.variant	1264	C A G T A T G A T G T T G T G A G T G A T C A T G T T A G C A A A G T T A C T T T T T C A A T C T
wsxfull.6.4.variant	1401	G A A T G A A A C C A A A C C T C G A G G A A A G T T T A C C T A T G A T G C A G T G T A C T G C T
wsxfull.12.1.variant	1314	G A A T G A A A C C A A A C C T C G A G G A A A G T T T A C C T A T G A T G C A G T G T A C T G C T
wsxfull.13.2.variant	1314	G A A T G A A A C C A A A C C T C G A G G A A A G T T T A C C T A T G A T G C A G T G T A C T G C T
wsxfull.6.4.variant	1451	G C A A T G A A C A T G A A T G C C A T C A T C G C T A T G C T G A A T T A T A T G T G A T T G A T
wsxfull.12.1.variant	1364	G C A A T G A A C A T G A A T G C C A T C A T C G C T A T G C T G A A T T A T A T G T G A T T G A T
wsxfull.13.2.variant	1364	G C A A T G A A C A T G A A T G C C A T C A T C G C T A T G C T G A A T T A T A T G T G A T T G A T
wsxfull.6.4.variant	1501	G T C A A T A T C A A T A T C T C A T G T G A A A C T G A T G G G T A C T T A A C T A A A A T G A C
wsxfull.12.1.variant	1414	G T C A A T A T C A A T A T C T C A T G T G A A A C T G A T G G G T A C T T A A C T A A A A T G A C
wsxfull.13.2.variant	1414	G T C A A T A T C A A T A T C T C A T G T G A A A C T G A T G G G T A C T T A A C T A A A A T G A C

FIG. 3E

wsxfull.6.4.variant	1551	T T G C A G A T G G T C A A C C A G T A C A A T C C A G T C A C T T G C G G A A A G C A C T T T G C
wsxfull.12.1.variant	1664	T T G C A G A T G G T C A A C C A G T A C A A T C C A G T C A C T T G C G G A A A G C A C T T T G C
wsxfull.13.2.variant	1664	T T G C A G A T G G T C A A C C A G T A C A A T C C A G T C A C T T G C G G A A A G C A C T T T G C
wsxfull.6.4.variant	1601	A A T T G A G G T A T C A T A G G A G C A G C C T T T A C T G T T C T G A T A T T C C A T C T A T T
wsxfull.12.1.variant	1514	A A T T G A G G T A T C A T A G G A G C A G C C T T T A C T G T T C T G A T A T T C C A T C T A T T
wsxfull.13.2.variant	1514	A A T T G A G G T A T C A T A G G A G C A G C C T T T A C T G T T C T G A T A T T C C A T C T A T T
wsxfull.6.4.variant	1651	C A T C C C A T A T C T G A G C C C A A A G A T T G C T A T T T G C A G A G T G A T G G T T T T A
wsxfull.12.1.variant	1564	C A T C C C A T A T C T G A G C C C A A A G A T T G C T A T T T G C A G A G T G A T G G T T T T A
wsxfull.13.2.variant	1564	C A T C C C A T A T C T G A G C C C A A A G A T T G C T A T T T G C A G A G T G A T G G T T T T A
wsxfull.6.4.variant	1701	T G A A T G C A T T T T C C A G C C A A T C T T C C T A T T A T C T G G C T A C A C A A T G T G G A
wsxfull.12.1.variant	1614	T G A A T G C A T T T T C C A G C C A A T C T T C C T A T T A T C T G G C T A C A C A A T G T G G A
wsxfull.13.2.variant	1614	T G A A T G C A T T T T C C A G C C A A T C T T C C T A T T A T C T G G C T A C A C A A T G T G G A
wsxfull.6.4.variant	1751	T T A G G A T C A A T C A C T C T C T A G G T T C A C T T G A C T C T C C A C C A A C A T G T G T C
wsxfull.12.1.variant	1664	T T A G G A T C A A T C A C T C T C T A G G T T C A C T T G A C T C T C C A C C A A C A T G T G T C
wsxfull.13.2.variant	1664	T T A G G A T C A A T C A C T C T C T A G G T T C A C T T G A C T C T C C A C C A A C A T G T G T C
wsxfull.6.4.variant	1801	C T T C C T G A T T C T G T G G T G A A G C C A C T G C C T C C A T C C A G T G T G A A A G C A G A
wsxfull.12.1.variant	1714	C T T C C T G A T T C T G T G G T G A A G C C A C T G C C T C C A T C C A G T G T G A A A G C A G A
wsxfull.13.2.variant	1714	C T T C C T G A T T C T G T G G T G A A G C C A C T G C C T C C A T C C A G T G T G A A A G C A G A

FIG. 3F



wsxfull.6.4.variant	1851	A A T T A C T A T A A A C A T T G G A T T A T T G A A A A T A T C T T G G G A A A A G C C A G T C T
wsxfull.12.1.variant	1764	A A T T A C T A T A A A C A T T G G A T T A T T G A A A A T A T C T T G G G A A A A G C C A G T C T
wsxfull.13.2.variant	1764	A A T T A C T A T A A A C A T T G G A T T A T T G A A A A T A T C T T G G G A A A A G C C A G T C T
wsxfull.6.4.variant	1901	T T C C A G A G A A T A A C C T T C A A T T C C A G A T T C G C T A T G G T T T A A G T G G A A A A
wsxfull.12.1.variant	1814	T T C C A G A G A A T A A C C T T C A A T T C C A G A T T C G C T A T G G T T T A A G T G G A A A A
wsxfull.13.2.variant	1814	T T C C A G A G A A T A A C C T T C A A T T C C A G A T T C G C T A T G G T T T A A G T G G A A A A
wsxfull.6.4.variant	1951	G A A G T A C A A T G G A A G A T G T A T G A G G T T T A T G A T G C A A A A T C A A A A T C T G T
wsxfull.12.1.variant	1964	G A A G T A C A A T G G A A G A T G T A T G A G G T T T A T G A T G C A A A A T C A A A A T C T G T
wsxfull.13.2.variant	1964	G A A G T A C A A T G G A A G A T G T A T G A G G T T T A T G A T G C A A A A T C A A A A T C T G T
wsxfull.6.4.variant	2001	C A G T C T C C C A G T T C C A G A C T T G T G T G C A G T C T A T G C T G T T C A G G T G C G C T
wsxfull.12.1.variant	1914	C A G T C T C C C A G T T C C A G A C T T G T G T G C A G T C T A T G C T G T T C A G G T G C G C T
wsxfull.13.2.variant	1914	C A G T C T C C C A G T T C C A G A C T T G T G T G C A G T C T A T G C T G T T C A G G T G C G C T
wsxfull.6.4.variant	2051	G T A A G A G G C T A G A T G G A C T G G G A T A T T G G A G T A A T T G G A G C A A T C C A G C C
wsxfull.12.1.variant	1964	G T A A G A G G C T A G A T G G A C T G G G A T A T T G G A G T A A T T G G A G C A A T C C A G C C
wsxfull.13.2.variant	1964	G T A A G A G G C T A G A T G G A C T G G G A T A T T G G A G T A A T T G G A G C A A T C C A G C C
wsxfull.6.4.variant	2101	T A C A C A G T T G T C A T G G A T A T A A A A G T T C C T A T G A G A G G A C C T G A A T T T T G
wsxfull.12.1.variant	2014	T A C A C A G T T G T C A T G G A T A T A A A A G T T C C T A T G A G A G G A C C T G A A T T T T G
wsxfull.13.2.variant	2014	T A C A C A G T T G T C A T G G A T A T A A A A G T T C C T A T G A G A G G A C C T G A A T T T T G

FIG. 3G

wsxfull.6.4.variant	2151	G A G A A T A A T T A A T G G A G A T A C T A T G A A A A G G A G A A A A A T G T C A C T T T A C
wsxfull.12.1.variant	2064	G A G A A T A A T T A A T G G A G A T A C T A T G A A A A G G A G A A A A A T G T C A C T T T A C
wsxfull.13.2.variant	2064	G A G A A T A A T T A A T G G A G A T A C T A T G A A A A G G A G A A A A A T G T C A C T T T A C
wsxfull.6.4.variant	2201	T T T G G A A G C C C C T G A T G A A A A A T G A C T C A T T G T G C A G T G T T C A G A G A T A T
wsxfull.12.1.variant	2114	T T T G G A A G C C C C T G A T G A A A A A T G A C T C A T T G T G C A G T G T T C A G A G A T A T
wsxfull.13.2.variant	2114	T T T G G A A G C C C C T G A T G A A A A A T G A C T C A T T G T G C A G T G T T C A G A G A T A T
wsxfull.6.4.variant	2251	G T G A T A A C C A T C A T A C T T C C T G C A A T G G A A C A T G G T C A G A A G A T G T G G G
wsxfull.12.1.variant	2164	G T G A T A A C C A T C A T A C T T C C T G C A A T G G A A C A T G G T C A G A A G A T G T G G G
wsxfull.13.2.variant	2164	G T G A T A A C C A T C A T A C T T C C T G C A A T G G A A C A T G G T C A G A A G A T G T G G G
wsxfull.6.4.variant	2301	A A A T C A C A C G A A A T T C A C T T T C C T G T G G A C A G A G C A A G C A C A T A C T G T T A
wsxfull.12.1.variant	2214	A A A T C A C A C G A A A T T C A C T T T C C T G T G G A C A G A G C A A G C A C A T A C T G T T A
wsxfull.13.2.variant	2214	A A A T C A C A C G A A A T T C A C T T T C C T G T G G A C A G A G C A A G C A C A T A C T G T T A
wsxfull.6.4.variant	2351	C G G T T C T G G C C A T C A A T T C A A T T G G T G C T T C T G T T G C A A A T T T A A T T T A
wsxfull.12.1.variant	2264	C G G T T C T G G C C A T C A A T T C A A T T G G T G C T T C T G T T G C A A A T T T A A T T T A
wsxfull.13.2.variant	2264	C G G T T C T G G C C A T C A A T T C A A T T G G T G C T T C T G T T G C A A A T T T A A T T T A
wsxfull.6.4.variant	2401	A C C T T T T C A T G G C C T A T G A G C A A A G T A A A T A T C G T G C A G T C A C T C A G T G C
wsxfull.12.1.variant	2314	A C C T T T T C A T G G C C T A T G A G C A A A G T A A A T A T C G T G C A G T C A C T C A G T G C
wsxfull.13.2.variant	2314	A C C T T T T C A T G G C C T A T G A G C A A A G T A A A T A T C G T G C A G T C A C T C A G T G C

FIG. 3H

wsxfull.6.4.variant	2451	T T A T C C C T T T A A A C A G C A G T T G T G T G A T T G T T T C C T G G A T A C T A T C A C C C A
wsxfull.12.1.variant	2364	T T A T C C C T T T A A A C A G C A G T T G T G T G A T T G T T T C C T G G A T A C T A T C A C C C A
wsxfull.13.2.variant	2364	T T A T C C C T T T A A A C A G C A G T T G T G T G A T T G T T T C C T G G A T A C T A T C A C C C A
wsxfull.6.4.variant	2501	G T G A T T A C A A G C T A A T G T A T T T T A T T A T T G A G T G G A A A A T C T T A A T G A A
wsxfull.12.1.variant	2414	G T G A T T A C A A G C T A A T G T A T T T T A T T A T T G A G T G G A A A A T C T T A A T G A A
wsxfull.13.2.variant	2414	G T G A T T A C A A G C T A A T G T A T T T T A T T A T T G A G T G G A A A A T C T T A A T G A A
wsxfull.6.4.variant	2551	G A T G G T G A A A T A A A A T G G C T T A G A A T C T C T T C A T C T G T T A A G A A G T A T T A
wsxfull.12.1.variant	2464	G A T G G T G A A A T A A A A T G G C T T A G A A T C T C T T C A T C T G T T A A G A A G T A T T A
wsxfull.13.2.variant	2464	G A T G G T G A A A T A A A A T G G C T T A G A A T C T C T T C A T C T G T T A A G A A G T A T T A
wsxfull.6.4.variant	2601	T A T C C A T G A T C A T T T T A T C C C C A T T G A G A A G T A C C A G T T C A G T C T T T A C C
wsxfull.12.1.variant	2514	T A T C C A T G A T C A T T T T A T C C C C A T T G A G A A G T A C C A G T T C A G T C T T T A C C
wsxfull.13.2.variant	2514	T A T C C A T G A T C A T T T T A T C C C C A T T G A G A A G T A C C A G T T C A G T C T T T A C C
wsxfull.6.4.variant	2651	C A A T A T T T A T G G A A G G A G T G G G A A A A C C A A A G A T A A T T A A T A G T T T C A C T
wsxfull.12.1.variant	2564	C A A T A T T T A T G G A A G G A G T G G G A A A A C C A A A G A T A A T T A A T A G T T T C A C T
wsxfull.13.2.variant	2564	C A A T A T T T A T G G A A G G A G T G G G A A A A C C A A A G A T A A T T A A T A G T T T C A C T
wsxfull.6.4.variant	2701	C A A G A T G A T A T T G A A A A A C A C C A G A G T G A T G C A G G T T T A T A T G T A A T T G T
wsxfull.12.1.variant	2614	C A A G A T G A T A T T G A A A A A C A C C A G A G T G A T G C A G G T T T A T A T G T A A T T G T
wsxfull.13.2.variant	2614	C A A G A T G A T A T T G A A A A A C A C C A G A G T G A T G C A G G T T T A T A T G T A A T T G T

FIG. 3I

wsxfull.6.4.variant	2751	G C C A G T A A T T A T T T C C T C T T C C A T C T T A T T G C T T G G A A C A T T A T T A A T A T
wsxfull.12.1.variant	2664	G C C A G T A A T T A T T T C C T C T T C C A T C T T A T T G C T T G G A A C A T T A T T A A T A T
wsxfull.13.2.variant	2664	G C C A G T A A T T A T T T C C T C T T C C A T C T T A T T G C T T G G A A C A T T A T T A A T A T
wsxfull.6.4.variant	2801	C A C A C C A A G A A T G A A A A G C T A T T T T G G A A G A T G T T C C G A A C C C C A A G
wsxfull.12.1.variant	2714	C A C A C C A A G A A T G A A A A G C T A T T T T G G A A G A T G T T C C G A A C C C C A A G
wsxfull.13.2.variant	2714	C A C A C C A A G A A T G A A A A G C T A T T T T G G A A G A T G T T C C G A A C C C C A A G
wsxfull.6.4.variant	2851	A A T T G T T C C T G G G C A C A A G G A C T T A A T T T T C A G A A G A G A C G G A C A T T T C T
wsxfull.12.1.variant	2764	A A T T G T T C C T G G G C A C A A G G A C T T A A T T T T C A G A A G A T G T T C C G A A C C C C
wsxfull.13.2.variant	2764	A A T T G T T C C T G G G C A C A A G G A C T T A A T T T T C A G A A G C C A G A A A C G T T T G A
wsxfull.6.4.variant	2901	T T G A A G T C T A A T C A T G A T C A C T A C A G A T G A A C C C A A T G T G C C A A C T T C C C
wsxfull.12.1.variant	2814	A A G A A T T G T T C C T G G G C A C A A G G A C T T A A T T T T C A G A A G A T G C T T G A A G G
wsxfull.13.2.variant	2814	G C A T C T T T T A T C A A G C A T A C A G C A T C A G T G A C A T G T G G T C C T C T T C T T T
wsxfull.6.4.variant	2951	A C A G T C T A T A G A G T A T T A G A A G A T T T T A C A T T T T G A A G A A G G C C G G A
wsxfull.12.1.variant	2864	C A G C A T G T T C G T T A A G A G T C A T C A C A C T C C C T A A T C T C A A G T A C C A G G
wsxfull.13.2.variant	2864	T G G A G C C T G A A A C A A T T T C A G A A G A T A T C A G T G T T G A T A C A T C A T G G A A A
wsxfull.6.4.variant	3001	A T T C
wsxfull.12.1.variant	2914	G A C A C A A C A C T G C G G A A G G C C A C A A G G T C C T C T G C A T A G G A A A C C A G A
wsxfull.13.2.variant	2914	A A T A A G A T G A G A T G A T G C C A C A C A C T G T G T C T C T C T A C T T T C A C A C A C

FIG. 3J

**FIG. 3K**

wsxfull.13.2.variant 3514 TTAGGAACCTTCTAGTAAGAAGACTTTTGCACTCTTACATGCCCTCAATTCCA  
wsxfull.13.2.variant 3564 AACTTGTCTACTCAGACTCATAGATCATGGAAACAAGATGTGTGACC  
wsxfull.13.2.variant 3614 TAACCTGTGTAAATTCACCTGAAGAAACCTTCAGATTTGTGTTATAATGGGT  
wsxfull.13.2.variant 3664 AATATAAGTGTAATAGATTATAGTTGTGGGTGGGAGAGAGAAAGAAAC  
wsxfull.13.2.variant 3714 CAGAGTCAAAATTTGAATAATAATTGTTCCAAATGAATGTGTCTGTTTGT  
wsxfull.13.2.variant 3764 CTCTCTTAGTAACATAGACAAATAATTGAGAAAGCCTTCATAAGCCTAC  
wsxfull.13.2.variant 3814 CAATGTAGACACGGCTCTCTATTTTATTTCCCAAGCTCTAGTGGGAAGGTC  
wsxfull.13.2.variant 3864 CCTTGTTTCCAGCTAGAAATAAGCCCAACAGACACCATCTTTGTGAGAT  
wsxfull.13.2.variant 3914 GTAAATTGTTTTTCAGAGGGCGTGTGTGTTTACCTCAAGTTTGTGTTG  
wsxfull.13.2.variant 3964 TACCACACACACACACACACATTCCTTAACACATGTCCTTGTGTGTTT  
wsxfull.13.2.variant 4014 TGAGAGTATATTATGTATTTATATTTGTGCTATCAGACTGTAGGATTTG  
wsxfull.13.2.variant 4064 AAGTAGGACTTTCCTAAATGTTTAAAGATAAACAGAAATTC

FIG. 3L

wsxfull.13.2.variant	1	M	I	C	Q	K	F	C	V	V	L	L	H	W	E	F	I	Y	V	I	T	A	F	N	L	S	Y	P	I	T	P	W	R	F	K	L	S	C	M	P	P	N	S	T	Y	D	Y	F	L	L	P		
mu.wsx.ecd	1	M	M	C	Q	K	F	Y	V	V	L	L	H	W	E	F	L	Y	V	I	A	A	L	N	L	A	Y	P	I	S	P	W	K	F	K	L	F	C	G	P	P	N	T	T	D	S	F	L	S	P			
wsxfull.13.2.variant	51	A	G	L	S	K	N	T	S	N	S	N	G	H	Y	E	T	A	V	E	P	K	F	N	S	S	G	T	H	F	S	N	L	S	K	T	T	F	H	C	C	F	R	S	E	Q	D	R	N	C	S		
mu.wsx.ecd	51	A	G	A	P	N	A	S	A	L	K	G	A	S	E	A	I	V	E	A	I	V	E	A	K	F	N	S	S	G	I	Y	V	P	E	L	S	K	T	V	F	H	C	C	F	G	N	E	Q	G	N	C	S
wsxfull.13.2.variant	101	L	C	A	D	N	I	E	G	K	T	F	V	S	T	V	N	S	L	V	F	Q	I	D	A	N	W	N	I	Q	C	W	L	K	G	D	L	K	L	F	I	C	Y	V	E	S	L	F	K	N			
mu.wsx.ecd	101	A	L	T	D	N	T	E	G	K	T	L	A	S	V	V	K	A	S	V	F	R	Q	L	G	V	N	W	D	I	E	C	W	M	K	G	D	L	T	L	F	I	C	H	M	E	P	L	P	K	N		
wsxfull.13.2.variant	151	L	F	R	N	Y	N	Y	K	V	H	L	L	Y	V	L	P	E	V	L	E	D	S	P	L	V	P	O	K	G	S	F	Q	M	V	H	C	N	C	S	V	H	E	C	C	E	C	L	V	P	V		
mu.wsx.ecd	151	P	F	K	N	Y	D	S	K	V	H	L	L	Y	D	L	P	E	V	I	D	S	P	L	P	P	L	K	D	S	F	Q	T	V	Q	C	N	C	S	L	R	G	-	C	E	C	H	V	P	V			
wsxfull.13.2.variant	201	P	T	A	K	L	N	D	T	L	L	M	C	L	K	I	T	S	G	G	V	I	F	Q	S	P	L	M	S	V	Q	P	I	N	M	V	K	P	D	P	P	L	G	L	H	M	E	I	T	D	D		
mu.wsx.ecd	200	P	R	A	K	L	N	Y	A	L	L	M	Y	L	E	I	T	S	A	G	V	S	F	Q	S	P	L	M	S	L	Q	P	M	L	V	V	K	P	D	P	P	L	G	L	H	M	E	V	T	D	D		
wsxfull.13.2.variant	251	G	N	L	K	I	S	W	S	S	P	P	L	V	P	F	P	L	Q	Y	Q	V	K	Y	S	E	N	S	T	T	V	I	R	E	A	D	K	I	V	S	A	T	S	L	L	V	D	S	I	L	P		
mu.wsx.ecd	250	G	N	L	K	I	S	W	D	S	Q	T	M	A	P	F	P	L	Q	Y	Q	V	K	Y	L	E	N	S	-	T	I	V	R	E	A	E	I	V	S	A	T	S	L	L	V	D	S	V	L	P			

FIG. 4A

wsxfull.13.2.variant	301	GSSYE	VQVRG	KRLDG	PGI	WSDWS	TPR	VFTTQ	DV	I	YFP	PKILT	SVGSN	V	SF															
mu.wsx.ecd	299	GSSYE	VQVRS	KRLDGS	G	VWSDWS	SPQ	VFTTQ	DVV	V	YFP	PKILT	SVGSN	A	SF															
wsxfull.13.2.variant	351	HCIYK	KENK	IVPSK	E	IVWW	MNLA	EKIP	QSQY	DV	VSD	H	VSKVT	F	NLNETK															
mu.wsx.ecd	349	HCIYK	KENQ	IVSSK	Q	IVWW	RNLA	EKIP	EIQYS	I	VSD	R	VSKVT	F	NLKATR															
wsxfull.13.2.variant	401	PRGKFTY	DAVYCCNE	HE	CHHRYA	EELV	IDVNI	INIS	CETD	GYLT	KMT	CRWS																		
mu.wsx.ecd	399	PRGKFTY	DAVYCCNE	QA	CHHRYA	EELV	IDVNI	INIS	CETD	GYLT	KMT	CRWS																		
wsxfull.13.2.variant	451	TSTIOSL	AESTL	QLRYH	RS	SLYC	S	D	PSI	HP	I	SEPK	DCY	LQSDG	F	YECIF														
mu.wsx.ecd	449	PSTIOSL	VGSTV	QLRYH	RC	SLYC	P	D	S	PSI	HP	T	SEPK	T	ASYRET	A	F	MNVFS												
wsxfull.13.2.variant	501	QPI	FLLSGYT	MWI	R	INHSL	GSLD	SPPT	CVLP	DSV	V	KPL	PPS	S	V	KAEIT	I	N												
mu.wsx.ecd	499	SQS	FYYLAI	QC	GF	R	INHSL	GSLD	SPPT	CVLP	DSV	V	KPL	PPS	N	V	KAEIT	V	N											
wsxfull.13.2.variant	551	I	GLLK	I	SWEK	P	VFP	ENNL	OFO	I	RYGL	SGKE	V	QWK	M	Y	E	V	Y	D	A	K	S	K	S	V	S	L	P	V
mu.wsx.ecd	549	T	GLLK	V	SWEK	P	VFP	ENNL	OFO	I	RYGL	SGKE	I	QWK	T	H	E	V	F	D	A	K	S	K	S	A	S	L	L	V

FIG. 4B



wsxfull.13.2.variant	601	P	D	L	C	A	V	V	A	V	Q	V	R	C	K	R	L	D	G	L	G	Y	W	S	N	W	S	N	P	A	Y	T	V	V	M	D	I	K	V	P	M	R	G	P	E	F	W	R	I	I	N
mu.wsx.ecd	599	S	D	L	C	A	V	V	V	V	Q	V	R	C	R	R	L	D	G	L	G	Y	W	S	N	W	S	S	P	A	Y	T	L	V	M	D	V	K	V	P	M	R	G	P	E	F	W	R	K	M	D

wsxfull.13.2.variant	651	G	D	T	M	K	K	E	K	N	V	T	L	L	W	K	P	L	M	K	N	D	S	L	C	S	V	O	R	Y	V	I	N	H	H	T	S	C	N	G	T	W	S	E	D	V	G	N	H	T	K
mu.wsx.ecd	649	G	D	V	T	K	K	E	R	N	V	T	L	L	W	K	P	L	T	K	N	D	S	L	C	S	V	R	R	Y	V	V	K	H	R	T	A	H	N	G	T	W	S	E	D	V	G	N	R	T	N

wsxfull.13.2.variant	701	F	T	F	L	W	T	E	Q	A	H	T	V	T	V	L	A	I	N	S	I	G	A	S	V	A	N	F	N	L	T	F	S	W	P	M	S	K	V	N	I	V	Q	S	L	S	A	Y	P	L	N
mu.wsx.ecd	699	L	T	F	L	W	T	E	P	A	H	T	V	T	V	L	A	V	N	S	L	G	A	S	L	V	N	F	N	L	T	F	S	W	P	M	S	K	V	S	A	V	E	S	L	S	A	Y	P	L	S

wsxfull.13.2.variant	751	S	S	C	V	I	V	S	W	I	L	S	P	S	D	Y	K	L	M	Y	F	I	E	W	K	N	L	N	E	D	G	E	I	K	W	L	R	I	S	S	S	V	K	K	Y	I	H	D	H
mu.wsx.ecd	749	S	S	C	V	I	L	S	W	I	L	S	P	D	D	Y	S	L	L	Y	L	V	I	E	W	K	I	L	N	E	D	D	G	M	K	W													

wsxfull.13.2.variant	801	F	I	P	I	E	K	Y	Q	F	S	L	Y	P	I	F	M	E	G	V	G	K	P	K	I	I	N	S	F	T	Q	D	D	I	E	K	H	O	S	D	A	G	L	Y	V	I	V	P	V	I	I
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wsxfull.13.2.variant	851	S	S	S	I	L	L	L	G	T	L	L	I	S	H	O	R	M	K	K	L	F	W	E	D	V	P	N	P	K	N	C	S	W	A	O	G	L	N	F	Q	K	P	E	T	F	E	H	L	F	I
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FIG. 4C

wsxfull.13.2.variant 901 KHTASVTCGPLLLEPETISEDISVDTSWKNKDEMMPTTVVSLSTTDLEK  
wsxfull.13.2.variant 951 GSVCI SDQFNSVNFSEAEGETEVTYEDESORQPFVKYATLISNSKPSETGE  
wsxfull.13.2.variant 1001 EQGLINSSVTKCFSSKN SPLKDSFSNSSWEIEAQAFFILSDQHPNII SPH  
wsxfull.13.2.variant 1051 LTFSEGLDELLKLEGNFPEENNDKKSIIYVLGVTSIKKRESGVLLTDKSRV  
wsxfull.13.2.variant 1101 SCPFPAPCLFTDIRVLQDSCSHFVENNINLGTSSKKTFASYMPQFQTCST  
wsxfull.13.2.variant 1151 QTHKIMENKMCDLTV

FIG. 4D

nu.wsx.ecd	1	GGGGCCCCCCTCGAAGTCGACGGTATCGATAAGCTTGATATCGAATTCCG
nu.wsx.ecd	51	GCCGGGACACAGGTGGGACACTCTTTTAGTCCTCAATCCCTGGCGGAGG
nu.wsx.ecd	101	CCACCCAGGCAACGCAGGACGCAGGGCGTTTGGGGACCAAGCAGCAGAC
nu.wsx.ecd	151	TGGGGCGGTACCTGCGGAGAGCCACGCACCTTCTCAGGCCCTCTGACTAC
nu.wsx.ecd	201	TTTGGAACCTGCCCGGGGCTGCGACATCAACCCCTTAAGTCCCGGAGGCG
nu.wsx.ecd	251	GAAAGAGGGTGGGTTGGTTTGAAAGACACAGGAAGAAATAATGTGCTGTG
nu.wsx.ecd	301	GGGCGGGTTAAGTTTCCCAACCCTCTTCCCCCTTCCCGAGCAATTAGAAA
nu.wsx.ecd	351	CAAAACAATAGAAAGCCAGCCCTCCGGCCACCAAGCAGCGGA
wsx.full.13.2.variant	1	.....GAAATTCGAGTCGAC

FIG. 5A

mu.wsx.ecd  
401 G C C C A A G C G G A G C C C A G C C G G A G C A C T C C T T A A A G G A T T T G C A G C G

wsx.full.13.2.variant 17 G G C G G C G T T A A G C T C T C G T G G C A T T A T C C T T C A G T G G G C T . . . A T T G

nu.wsx.ecd  
451 GTGAGGAA A A A C C A G A C C G A C C G A G G A A T C C A G G T G

wsx.full.13.2.variant  
64 G A C T G A C T T T C T T A T G C T G G G A T G T G . . . C C T T A G A G G A T T A T G G G T G

[illegible]

mu.wsx.ecd  
551 C A C T G G G A A T T T C T T T A T G T G A T A G C T G C A C T T A A C C T G G C A T A T C C A A T

wsx.full.13.2.variant  
160 C A T T G G G A A T T T A T T T A T G T G A T A A C T G C G T T T A A C T T G T C A T A T C C A A T

601	CTCTCCCTGGGA	AATTTAAGTGT	TTTGTGG	CCACCG	AACA	CAACCG	ATG
wsx.full.13.2.variant	TACTCCCTGGGA	GATTTAAGTGT	CTTGTG	CCACCG	AATT	CAACCT	ATG

[illegible]

**FIG. 5B**

mu.wsx.ecd	701	GGGGC	TTCTGA	AGCAAT	TGTTGAAG	CTAAAG	TTTAATTCAAGTGGTA	TCTA
	310	GGACA	TTATGA	GACAGC	TGTTGAAC	CTAAAG	TTTAATTCAAGTGGTA	CTCA
mu.wsx.ecd	751	CGTT	CTCTGA	GTATCCAA	AACAAGTCT	TTCCACTGTTGCTTT	GGAA	TGAGC
	360	CTTT	CTCTAA	CTATCCAA	AACAAC	TTCCACTGTTGCTTT	GGAG	TGAGC
mu.wsx.ecd	401	AAGG	TCAA	AACTGCTCT	IGCACTCA	CAGACA	AACA	CTG
	410	AAGA	TAGAA	AACTGCTCT	CTTATGTG	CAGACA	AACA	TGAGCATTT
mu.wsx.ecd	451	GCTT	CAAGT	GAAGG	CTTCA	GTTTTT	CCAGCT	GGGA
	460	GTTT	CAACAGT	AAATTT	CTTCTT	AGTTTTT	CAACA	TAAGTGGAA
mu.wsx.ecd	501	CATA	GAGTGCT	GGATGA	AAAGGG	GACTTG	CAATTATT	CATCTGTGG
	510	CATA	CAGTGCT	GGCTTA	AAAGGGA	GACTTA	CAATTATT	CATCTGTGG
mu.wsx.ecd	551	AGC	CATT	ACCTA	AGAA	CCCTTCA	AGAA	TATGACTCT
	560	AGT	CATT	ATTA	TAAGAA	CTATTC	AGAA	TATGACTCT

FIG. 5C

mu.wsx.ecd	1001	T T A T A T G A	T C T G C C C T G A A G T C A	T A G A T	G A T T C G	C C T C T G	C C C C A C	T G A A
wsx.full.13.2.variant	610	T T A T A T G T	T C T G C C C T G A A G T G T	T A G A A	G A T T C A	C C T C T G	T T C C C A	A A A
mu.wsx.ecd	1051	A G A C A G C	T T T C A G A C T G T C C A A	T G C A A C	T G C A G T	C T T C G G G	- - -	G A T G T G
wsx.full.13.2.variant	660	A G C A G T	T T T C A G A T G G T T C A C T	T G C A A T	T G C A G T	G T T C A T	G A A T	G T T G T G
mu.wsx.ecd	1098	A A T G T C A	T G T G C C A G T A C C C A	G A G C C A A A C T C A A C T	A C G C T C T	C T C T	C T G	A T G
wsx.full.13.2.variant	710	A A T G T C T	T G T G C C T G T G C C A A	C A G C C A A A C T C A A C G A C	A C T C T	C T C T	C T T	A T G
mu.wsx.ecd	1148	T A T T T G G	A A A T C A C A T C T G C C G G T	G T G A	G T T T	C A G T C A C C T C T	G A T G T C	
wsx.full.13.2.variant	760	T G T T T G A	A A A T C A C A T C T G T G G A	G T A A	T T T T	C A G T C A C C T C T	A A T G T C	
mu.wsx.ecd	1198	A C T G C A G C C C C A T	G C T T G T T G T G A A A C C C	G A T C C A C C	C T T A G G T T T G C A T A			
wsx.full.13.2.variant	810	A G T T C A G C C C C A T	A A A T A T G T G A A G C C T	G A T C C A C C A	T T A G G T T T G C A T A			
mu.wsx.ecd	1248	T G G A A G T	C A C A G A T G A T G G T A A T T T A A A G A T T T C T T G G G A	C A G C C A	A A C A			
wsx.full.13.2.variant	860	T G G A A A T	C A C A G A T G A T G G T A A T T T A A A G A T T T C T T G G T C C A G C C C A C C A					

FIG. 5D

mu.wsx.ecd wsxfull.13.2.variant	1298	A	T	G	G	C	A	C	C	A	T	T	T	C	C	G	C	T	T	C	A	A	T	A	T	T	T	A	G	A	A	T	T	C	T	A	C									
	910	T	T	G	G	T	A	C	C	A	T	T	T	C	C	A	C	T	T	C	A	A	T	A	T	T	C	A	G	T	G	A	A	T	T	C	A	C								
mu.wsx.ecd wsxfull.13.2.variant	1348	A	A	-	-	-	T	T	G	T	A	A	G	A	G	A	G	C	T	G	A	A	A	T	T	G	T	C	T	C	A	G	C	T	A	C	T	C	T	A	C					
	960	A	A	C	A	G	T	T	A	T	A	T	C	A	G	A	A	G	C	T	G	A	C	A	A	G	A	T	T	G	T	C	T	C	A	G	C	T	A	C	T	A	C			
mu.wsx.ecd wsxfull.13.2.variant	1395	T	A	G	A	C	A	G	T	G	T	G	C	T	T	C	C	T	G	G	A	T	C	T	T	C	A	T	A	T	G	A	G	G	T	C	A	G	C	A	A	G				
	1010	T	A	G	A	C	A	G	T	A	T	A	C	T	T	C	C	T	G	G	A	T	C	T	T	C	G	T	A	T	G	A	G	G	T	C	A	G	C	A	A	G				
mu.wsx.ecd wsxfull.13.2.variant	1445	A	G	A	C	T	G	G	A	T	G	G	T	T	C	A	G	G	A	G	T	C	T	G	G	A	G	T	T	C	A	C	C	T	C	A	A	G	T	C	T	T				
	1060	A	G	A	C	T	G	G	A	T	G	G	T	T	C	A	G	G	A	A	T	C	T	G	G	A	G	T	A	C	T	C	C	T	C	G	T	C	T	T	T					
mu.wsx.ecd wsxfull.13.2.variant	1495	T	A	C	C	A	C	A	A	G	A	T	G	T	G	T	G	T	A	T	T	T	C	C	A	C	C	C	C	A	A	A	A	T	T	C	T	G	A	C	T	A	G	T	T	G
	1110	T	A	C	C	A	C	A	A	G	A	T	G	T	C	A	T	A	T	A	C	T	T	T	C	C	A	C	C	T	A	A	A	A	T	T	C	T	G	A	C	A	A	G	T	T
mu.wsx.ecd wsxfull.13.2.variant	1545	G	A	T	C	G	A	A	T	G	C	T	T	C	C	T	T	T	C	A	T	T	G	C	A	T	T	G	C	A	A	A	A	A	C	C	A	G	A	T	T	G	T	C		
	1160	G	G	T	C	T	A	A	T	G	T	T	T	C	T	T	T	C	A	C	T	G	C	A	T	A	A	G	A	A	A	A	C	A	A	G	A	A	T	T	G	T	T	T		

FIG. 5E

mu.wsx.ecd	1595	T C C T C A A A A	C A G A T	A G T T T G G T G G A	G G A A T C	T A G C T G A G A A A A T	C C C T G A
	1210	C C C T C A A A A	A G A T T	T G T T T G G T G G A	T G A A T T	T A G C T G A G A A A A T	T C C T C A
mu.wsx.ecd	1645	G A T A C A G T A	C A G C A	T T G T G A G T G A C	C G A G T T A G C A A A G T T A C	C T T C T C C A	
	1260	A A G C C A G T A	T G A T G	T T G T G A G T G A T	C A T G T T A G C A A A G T T A C	T T T T T C A	
mu.wsx.ecd	1695	A C C T G A A A	A G C C A C C A	G A C C T C G A G G	G A A G T T T A C C T A T G A C	G C A G T G T A C	
	1310	A T C T G A A T	G A A A C C A A	A C C T C G A G G	A A G T T T A C C T A T G A T	G C A G T G T A C	
mu.wsx.ecd	1745	T G C T G C A A T	G A G C A G G	C G T G C C A T C A C	C G C T A T G C T G A A T T A T A	C G T G A T	
	1360	T G C T G C A A T	G A A C A T G	A T G C C A T C A T	C G C T A T G C T G A A T T A T A	T G T G A T	
mu.wsx.ecd	1795	C G A T G T C A A T	A T C A A T A T	A T C A T G T G A A A C T G A C	G G G T A C T T A A C T A A A A		
	1410	T G A T G T C A A T	A T C A A T A T	C T C A T G T G A A A C T G A T	G G G T A C T T A A C T A A A A		
mu.wsx.ecd	1845	T G A C T T G C A G A T	G G T C A C	C C A G C A C A A T C C A A	A T C A C T A G T G G G	A A G C A C T	
	1460	T G A C T T G C A G A T	G G T C A C	C C A G T A C A A T C C A G	T C A C T T G C G G A A A G C A C T		

FIG. 5F





mu.wsx.ecd	2194	AGTCTTTCCG	GAGAAATAACCTTCAATTCCAGATTTCG	ATATGGC	TTAAGTG
wsx.full.13.2.variant	1809	AGTCTTTCC	GAGAAATAACCTTCAATTCCAGATTTCG	CTATGGT	TTAAGTG
mu.wsx.ecd	2244	GAAAGAA	TACAATGGAAGA	CACATGAGGTTAT	TCAAGA
wsx.full.13.2.variant	1859	GAAAGAA	TACAATGGAAGA	TGTATGAGGTTAT	ATCAAAA
mu.wsx.ecd	2294	TCTGC	CAGCTCT	GCTGCTGT	CAGAGCTT
wsx.full.13.2.variant	1909	TCTGT	CAGTCT	CCAGTTC	CAGACTT
mu.wsx.ecd	2344	TGCTG	CCGGC	GGTCT	GGTGGT
wsx.full.13.2.variant	1959	GCCTGT	AGAGGCT	AGATGGACT	GGATATTGGAGTAATTGGAGCAATC
mu.wsx.ecd	2394	CAGCCTA	TACGC	TTGTTCATGGAT	GATGAGAGG
wsx.full.13.2.variant	2009	CAGCCTA	CACAG	TTGTTCATGGAT	ATGAGAGG
mu.wsx.ecd	2444	TTTTGGAGAA	AATGGATGGG	GACGT	TAATAAAGGAGAG
wsx.full.13.2.variant	2059	TTTTGGAGAA	TAAATTAATGGA	GATAC	TGATAAAGGAGAA

FIG. 5H

ml.wsx.ecd	2494	C	T	T	G	C	T	T	T	G	G	A	A	G	C	C	C	T	G	A	C	G	A	A	A	A	T	G	A	C	T	G	T	G	T	G	A	G	A																	
wsx.full.13.2.variant	2109	T	T	A	C	T	T	T	G	G	A	A	G	C	C	C	T	G	A	T	G	A	A	A	A	A	T	G	A	C	T	G	T	G	C	A	G	T	G	T	C	A	G	A												
ml.wsx.ecd	2544	G	G	T	A	C	G	T	G	T	G	A	A	G	C	A	T	C	G	T	A	C	T	G	C	C	A	C	A	A	T	G	G	A	C	A	T	G	G	T	C	A	G	A	A	G	A	T								
wsx.full.13.2.variant	2159	G	A	T	A	T	G	T	G	A	T	A	A	C	C	A	T	C	A	T	A	C	T	T	C	C	T	G	C	A	A	T	G	G	A	A	C	A	T	G	G	T	C	A	G	A	A	G	A	T						
ml.wsx.ecd	2594	G	T	G	G	G	A	A	A	T	C	G	G	A	C	A	A	T	C	A	C	T	T	T	C	C	T	G	T	G	G	A	C	A	G	A	C	A	C	A	C	A	C	A	C	A	C									
wsx.full.13.2.variant	2209	G	T	G	G	G	A	A	A	T	C	A	C	A	C	A	A	T	T	C	A	C	T	T	C	C	T	G	T	G	G	A	C	A	G	A	C	A	G	C	A	A	G	C	A	C	A	T	A	C						
ml.wsx.ecd	2644	T	G	T	T	A	C	A	G	T	T	C	T	G	G	C	T	G	T	C	A	A	T	T	C	C	C	T	C	C	T	T	C	C	T	T	G	T	G	T	G	C	A	A	A	T	T	T	A							
wsx.full.13.2.variant	2259	T	G	T	T	A	C	G	G	T	T	C	T	G	G	C	C	A	T	C	A	A	T	T	C	C	A	T	T	G	G	T	G	C	T	T	C	T	G	T	G	C	A	A	A	T	T	T	A							
ml.wsx.ecd	2694	A	C	C	T	T	A	C	C	T	T	C	T	C	A	T	G	G	C	C	C	A	T	G	A	G	T	A	A	A	G	T	G	A	G	T	G	C	T	G	T	G	C	A	G	T	C	A	C	T	C					
wsx.full.13.2.variant	2309	A	T	T	A	A	C	C	T	T	T	C	A	T	G	G	C	C	T	A	T	G	A	G	C	A	A	A	G	T	A	A	T	A	T	C	G	T	G	C	A	G	T	C	A	C	T	C								
ml.wsx.ecd	2744	A	G	T	G	C	T	T	A	T	C	C	C	C	T	G	A	G	C	A	G	C	A	G	C	A	T	T	G	T	G	T	C	A	T	C	C	T	T	C	C	T	T	C	C	T	G	T	C	A	C	T	G	T	C	
wsx.full.13.2.variant	2359	A	G	T	G	C	T	T	A	T	C	C	T	T	A	A	A	C	A	G	C	A	G	T	T	G	T	G	T	G	A	T	G	A	T	T	C	C	T	T	C	C	T	G	G	A	T	A	C	T	A	C	T	A	T	C

FIG. 5I

nu.wsx.ecd	2794	ACC	TGA	TGAT	TAT	AGT	CTGT	TAT	TAT	CTGG	TTAT	TGAA	TGGA	AGAT	CTTA																																			
	2409	ACC	CAG	TGAT	TAC	AAG	CTAAT	GAT	TAT	TTAT	TGAA	TGGA	AAAT	CTTA																																				
nu.wsx.ecd	2844	ATGA	AGAT	GA	TGG	AAT	GAAG	TGGCT																																										
	2459	ATGA	AGAT	GG	TGA	AAT	AAAT	ATGGCT	TAGA	ATCTCT	TCA	TCTGT	TAA	GAA	G																																			
wsxfull.13.2.variant	2509	TAT	TAT	ATC	CAT	GAT	CA	T	T	T	ATC	CCCAT	TG	AGA	AGTAC	CA	GTCT																																	
wsxfull.13.2.variant	2559	T	A	C	C	C	A	A	T	A	T	T	A	T	G	G	A	A	A	C	C	A	A	G	A	T	A	A	T	A	A	T	A	G	T	T														
wsxfull.13.2.variant	2609	T	C	A	C	T	C	A	A	G	A	T	G	A	A	A	C	A	C	C	A	G	A	G	T	G	C	A	G	G	T	T	A	T	A	T	G	T	A											
wsxfull.13.2.variant	2659	A	T	T	G	T	G	C	C	A	G	T	A	A	T	T	A	T	T	C	C	T	C	T	T	C	C	A	T	T	A	T	T	G	C	T	T	G	G	A	A	C	A	T	T	A	T	T		
wsxfull.13.2.variant	2709	A	A	T	A	T	C	A	C	A	C	C	A	A	A	A	G	A	A	T	G	A	A	A	A	G	C	T	A	T	T	T	G	G	G	A	A	G	A	T	G	T	T	C	C	G	A	A	C	C
wsxfull.13.2.variant	2759	C	C	A	A	G	A	A	T	T	G	T	C	C	T	G	G	G	C	A	C	A	A	G	G	A	C	T	A	A	T	T	T	C	A	G	A	A	G	C	C	A	G	A	A	C	C			

FIG. 5J

wsxfull.13.2.variant 2809 T T T G A G C A T C T T T T A T C A A G C A T A C A G C A T C A G T G A C A T G T G G T C C T C T

wsxfull.13.2.variant 2859 T C T T T T G G A G C C T G A A C A A T T T C A G A A G A T A T C A G T G T T G A T A C A T C A T

wsxfull.13.2.variant 2909 G G A A A A T A A A G A T G A G A T G A T G C C A A C A A C T G T G G T C T C T A C T T T C A

wsxfull.13.2.variant 2959 A C A A C A G A T C T T G A A A G G G T T C T G T T T G T A T T A G T G A C C A G T T C A A C A G

wsxfull.13.2.variant 3009 T G T T A A C T T C T G A G G C T G A G G T A C T G A G G T A A C C T A T G A G G A C G A A A

wsxfull.13.2.variant 3059 G C C A G A G A C A A C C C T T T G T T A A T A C G C C A C G C T G A T C A G C A A C T C T A A A

wsxfull.13.2.variant 3109 C C A A G T G A A A C T G G T G A A G A A C A A G G G C T T A T A A T A G T T C A G T C A C C A A

wsxfull.13.2.variant 3159 G T G C T T C T C T A G C A A A A T T C T C G T T G A A G G A T T C T T T C T A A T A G C T

wsxfull.13.2.variant 3209 C A T G G G A G A T A G A G G C C C A G G C A T T T T T A T A T T A T C A G A T C A G C A T C C C

FIG. 5K

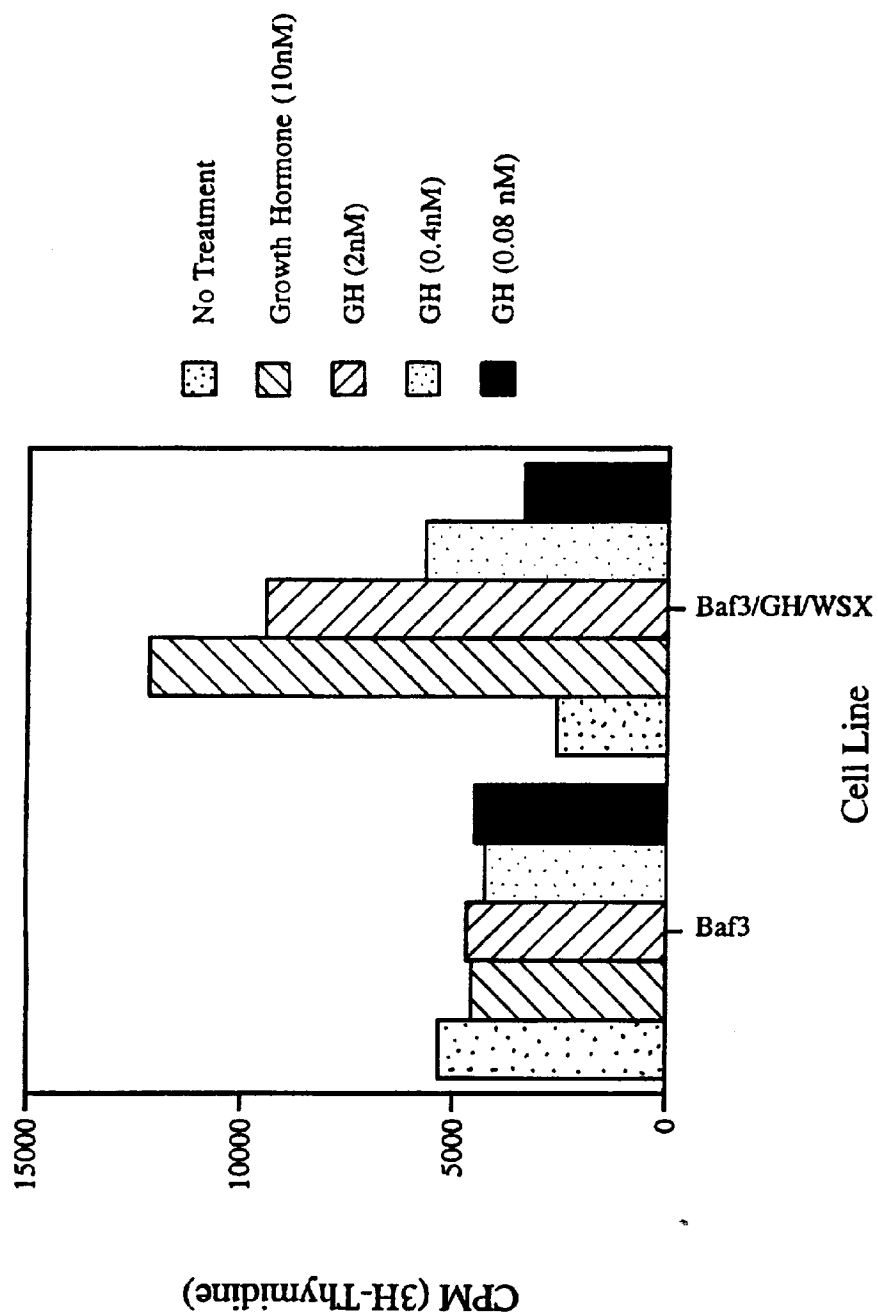
wsxfull.13.2.variant 3259 A A C A T A A T T C A C C A C A C C T C A C A T T C T C A G A A G G A T T G G A T G A A C T T T T  
wsxfull.13.2.variant 3309 G A A A T T G G A G G G A A T T T C C C T G A A G A A A A T A A T G A T A A A A G T C T A T C T  
wsxfull.13.2.variant 3359 A T T A T T A G G G G T C A C C T C A A T C A A A A G A G A G A G A G T G G T G T G C T T T T G  
wsxfull.13.2.variant 3409 A C T G A C A A G T C A A G G G T A T C G T G C C C A T T C C C A G C C C C C T G T T A T T C A C  
wsxfull.13.2.variant 3459 G G A C A T C A G A G T T C T C C A G G A C A G T T G C T C A C A C T T T G T A G A A A T A A T A  
wsxfull.13.2.variant 3509 T C A A C T T A G G A A C T T C T A G T A A G A A G A C T T T T G C A T C T T A C A T G C C T C A A  
wsxfull.13.2.variant 3559 T T C C A A A C T T G T T C T A C T C A G A C T C A T A A G A T C A T G G A A A A C A A G A T G T G  
wsxfull.13.2.variant 3609 T G A C C T A A C T G T G T A A T T T C A C T G A A G A A A C C T T C A G A T T T G T G T T A T A A  
wsxfull.13.2.variant 3659 T G G G T A A T A T A A A G T G T A A T A G A T T A T A G T T G T G G G A G A G A G A A A A

**FIG. 5L**

wsxfull.13.2.variant 3709 G A A C C A G A G T C A A A T T G A A A A T A A T T G T T C C A A A T G A A T G T T G T C T G T  
wsxfull.13.2.variant 3759 T T G T T C T C T T A G T A C A T A G A C A A A A A T T T G A G A A A G C C T T C A T A A G  
wsxfull.13.2.variant 3809 C C T A C C A A T G T A G A C A C G C T C T T C T A T T T A T T C C C A A G C T C T A G T G G G A  
wsxfull.13.2.variant 3859 A G G T C C C T T G T T T C C A G C T A G A A A T A A G C C C A A C A G A C A O C A T C T T T T G T  
wsxfull.13.2.variant 3909 G A G A T G T A A T T G T T T T T C A G A G G G C G T G T T G T T T A C C T C A A G T T T T G  
wsxfull.13.2.variant 3959 T T T T G T A C C A C A C A C A C A C A C A C A T T C T T A A C A C A T G T C C T T G T G  
wsxfull.13.2.variant 4009 T G T T T T G A G A G T A T A T T A T G T A T T T A T A T T T T G T G C T A T C A G A C T G T A G G  
wsxfull.13.2.variant 4059 A T T T G A A G T A G G A C T T T C C T A A A T G T T T A A G A T A A C A G A A T T C

FIG. 5M

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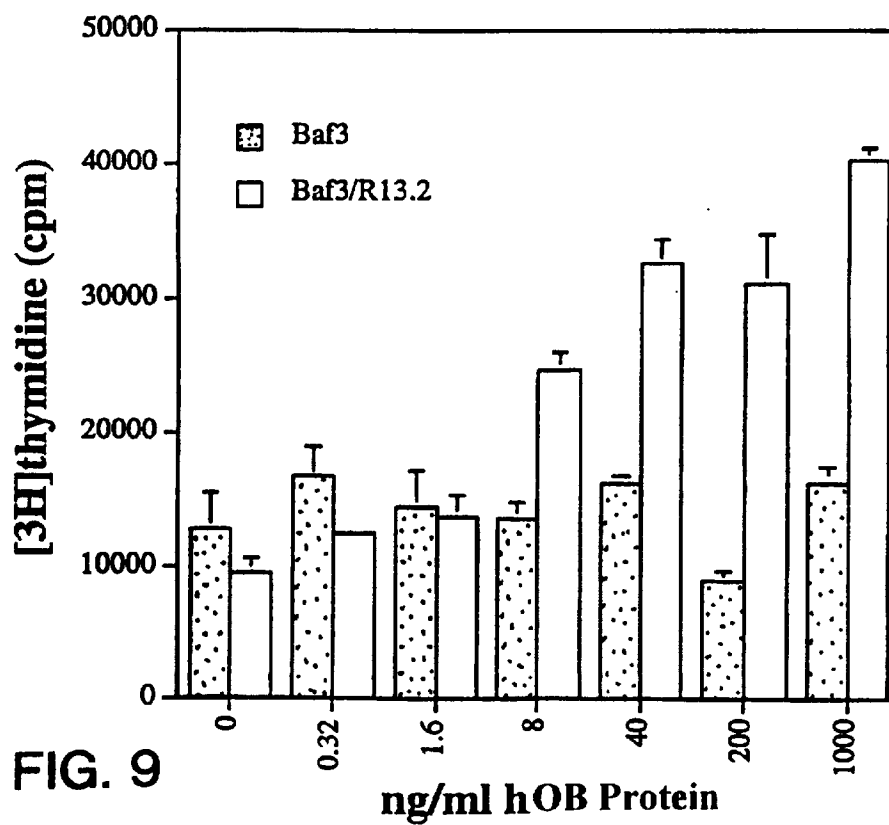
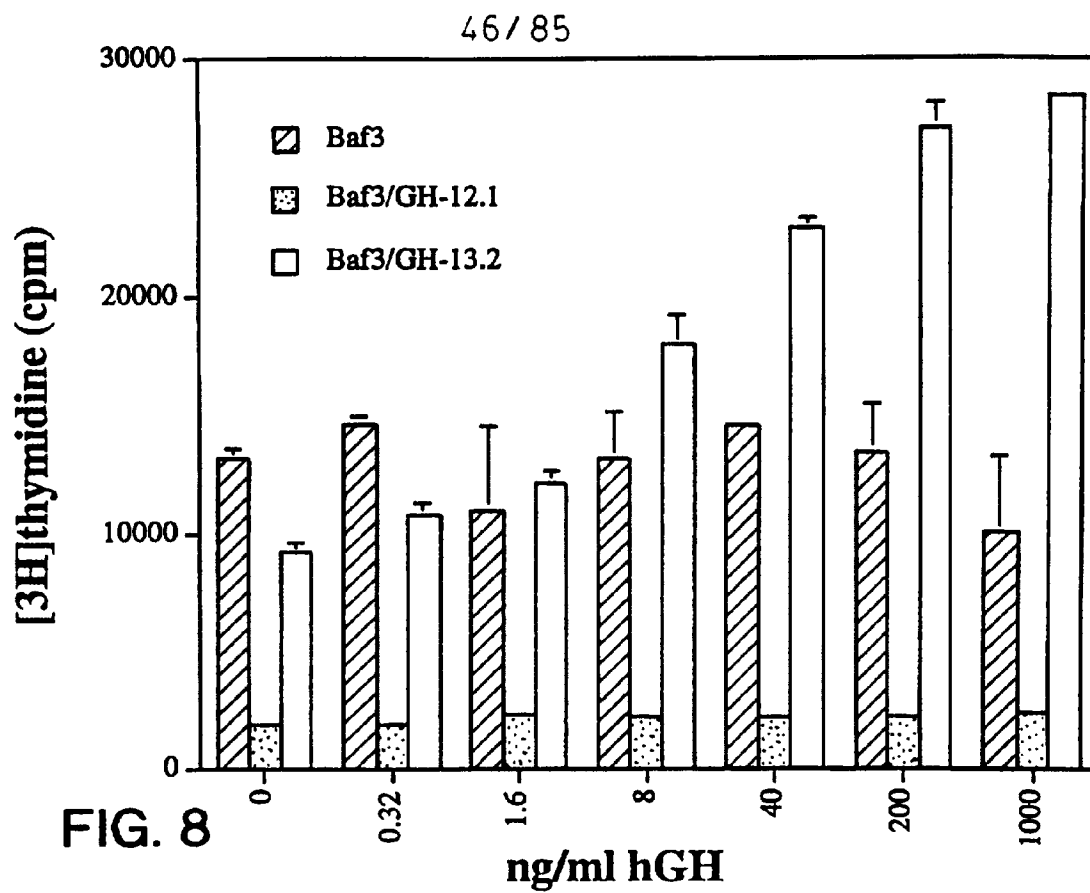
Cell Line  
**FIG. 6**



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<b>Murine</b>			
-213	Sense:	GGGTTAAGTTTCCCACCC	(SEQ ID NO:9)
	Antisense:	GGGTGGGAACTTAACCC	(SEQ ID NO:10)
	Scrambled:	AGGATACAGTGGGATCCC	(SEQ ID NO:11)
-99	Sense:	GCCCCGAGCACTCCTTTAA	(SEQ ID NO:12)
	Antisense:	TTAAAGGAGTGCTCCCGC	(SEQ ID NO:13)
	Scrambled:	GAGCGGCCCTGTTAGATA	(SEQ ID NO:14)
-20	Sense:	GTATACACCTCTGAAGAA	(SEQ ID NO:15)
	Antisense:	TTCTTCAGAGGTGTACAC	(SEQ ID NO:16)
	Scrambled:	ATGCGAGGCTACTTCTAT	(SEQ ID NO:17)
+84	Sense:	CTCTCCCTGGAAATTTAA	(SEQ ID NO:18)
	Antisense:	TTAAATTTCCAGGGAGAG	(SEQ ID NO:19)
	Scrambled:	ATTTGAAGGAGTTAAGCC	(SEQ ID NO:20)
+211	Sense:	AATTTAATTCAAGTGGTA	(SEQ ID NO:21)
	Antisense:	TACCAGTTGAATTAAATT	(SEQ ID NO:22)
	Scrambled:	GTATCACTTCATAATATA	(SEQ ID NO:23)
<b>Human</b>			
5L	Sense:	GATGGTCAGGGTGAAGTG	(SEQ ID NO:24)
	Antisense:	CAGTTCACCCGTGACCATC	(SEQ ID NO:25)
	Scrambled:	GAGGCGAATGTGCGGATT	(SEQ ID NO:26)
+85	Sense:	CTTAAATCTCCAAGGAGT	(SEQ ID NO:27)
	Antisense:	ACTCCTTGGAGATTTAAG	(SEQ ID NO:28)
	Scrambled:	AAGTCTTAAGCCAGACTT	(SEQ ID NO:29)
-47	Sense:	TCTAAGGCACATCCCAGC	(SEQ ID NO:30)
	Antisense:	GCTGGGATGTGCCTTAGA	(SEQ ID NO:31)
	Scrambled:	CGCAATGAATTGACCCCC	(SEQ ID NO:32)
-20	Sense:	TACTTCAGAGAAGTACAC	(SEQ ID NO:33)
	Antisense:	GTGTACTTCTCTGAAGTA	(SEQ ID NO:34)
	Scrambled:	GAATCACGGTAACTATCA	(SEQ ID NO:35)
+185	Sense:	CAGCTGTCTCATAATGTC	(SEQ ID NO:36)
	Antisense:	GACATTATGAGACAGCTG	(SEQ ID NO:37)
	Scrambled:	TTCGTCAAGCCATCTGAT	(SEQ ID NO:38)

FIG. 7



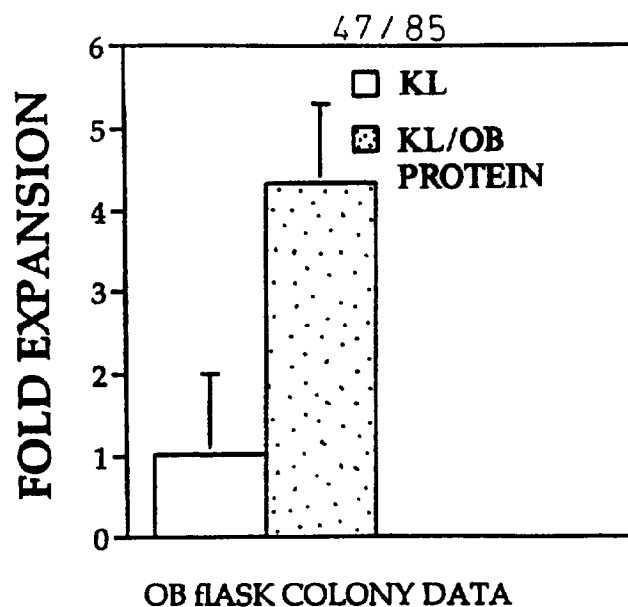


FIG. 10A

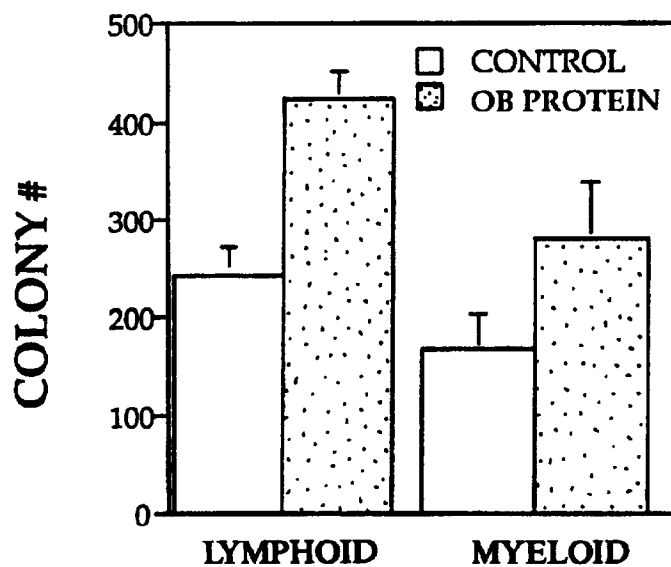


FIG. 10B

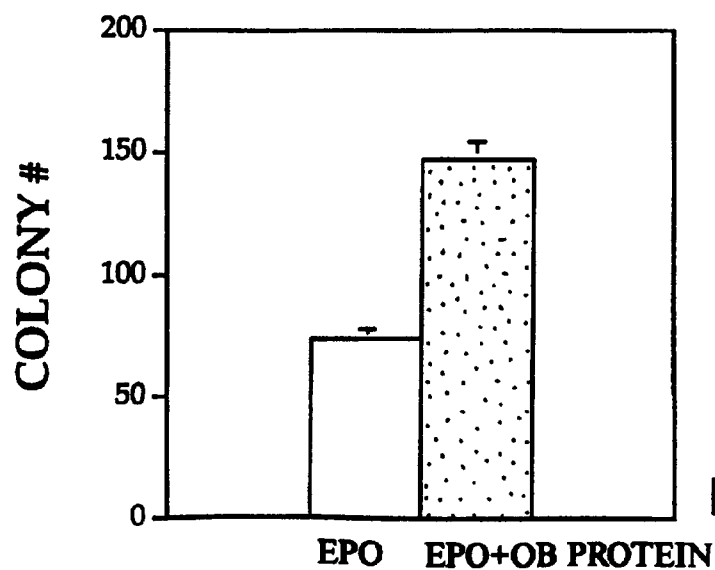


FIG. 10C

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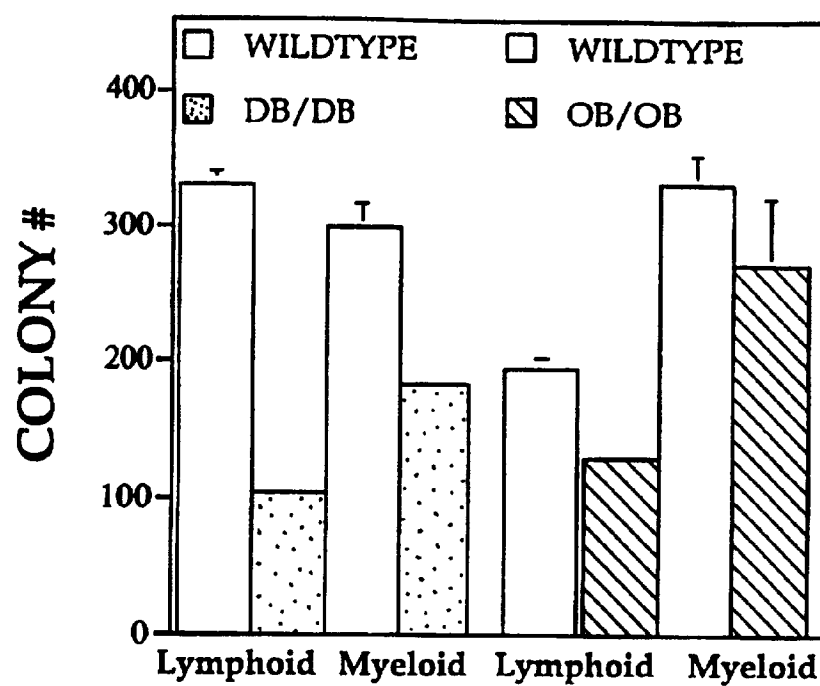


FIG. 11

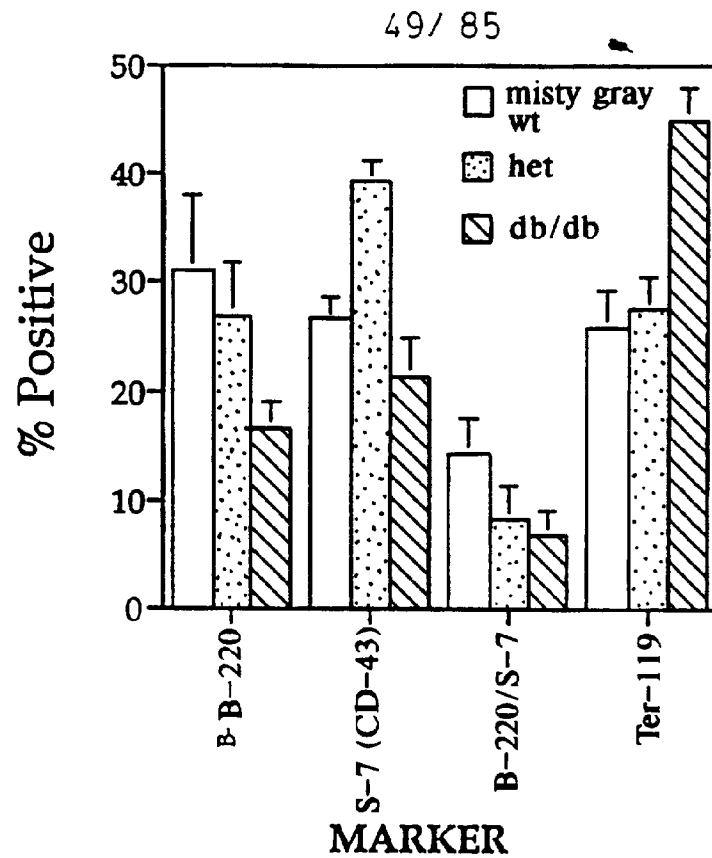


FIG. 12A

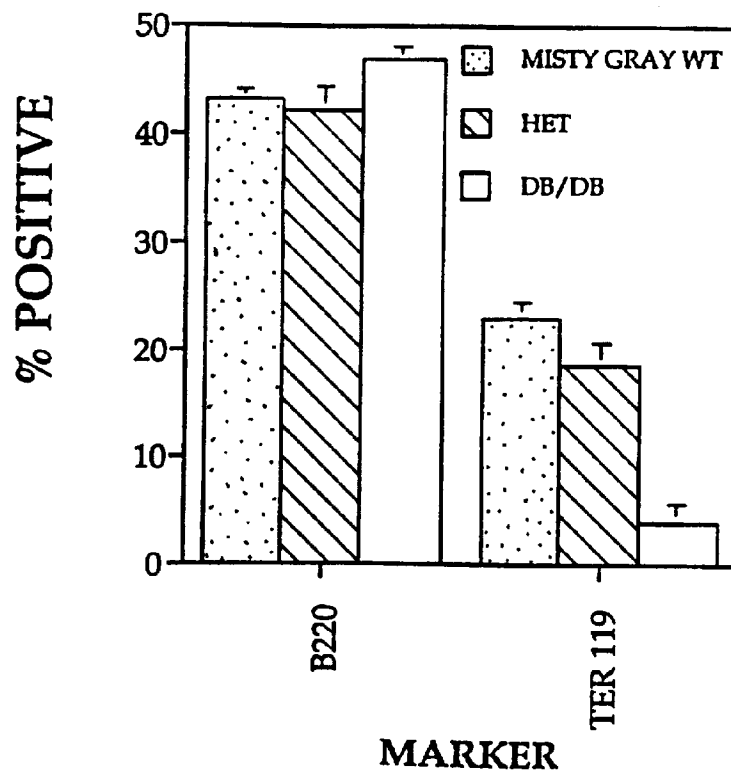


FIG. 12B

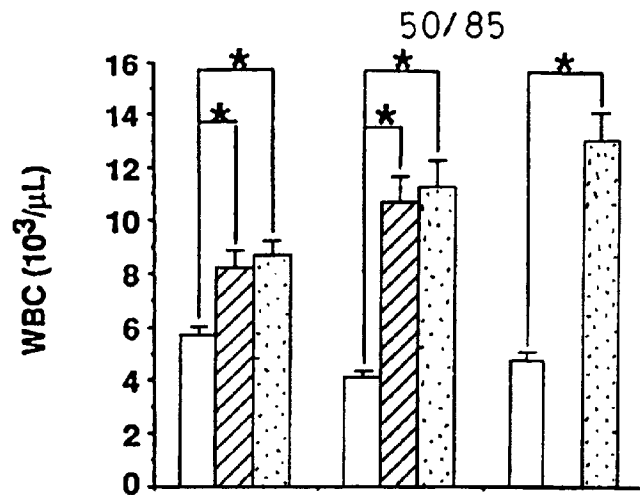


FIG. 13A

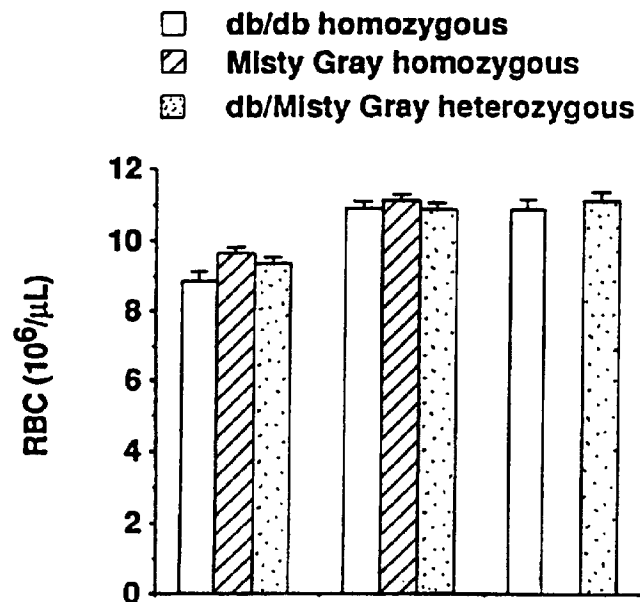


FIG. 13B

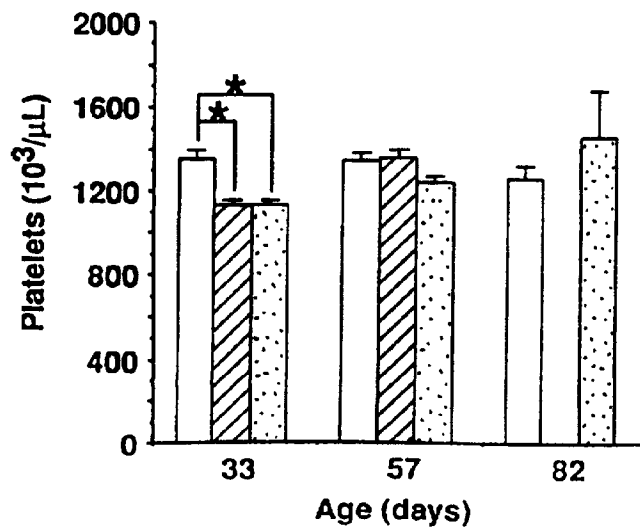


FIG. 13C

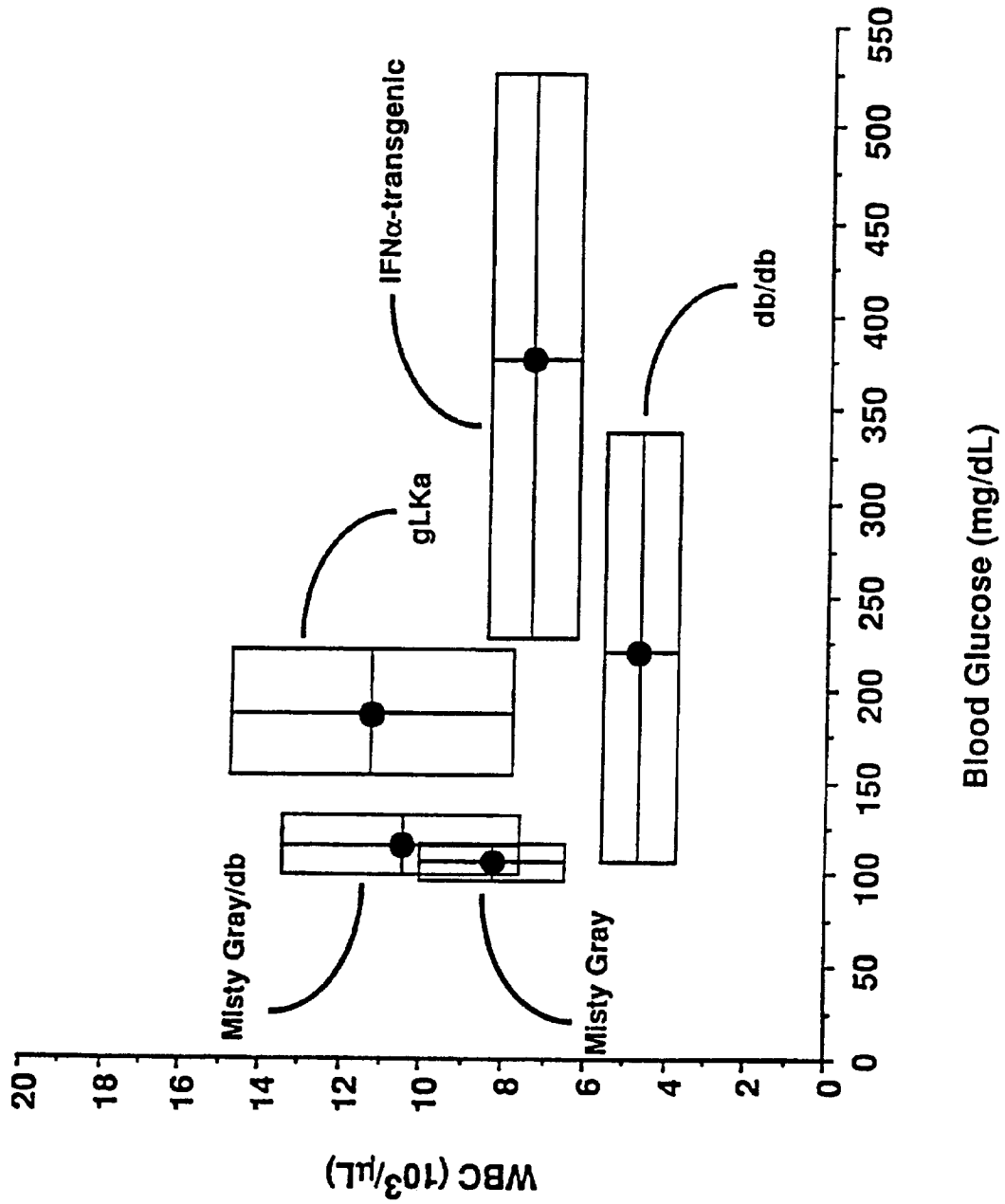


FIG. 14

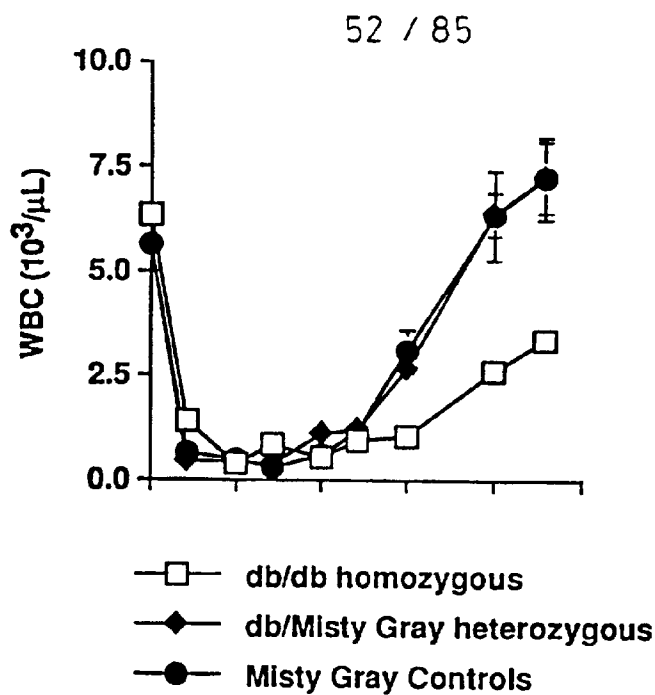


FIG. 15A

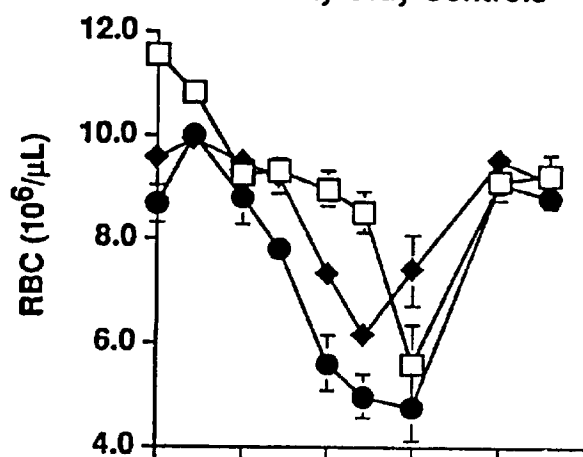


FIG. 15B

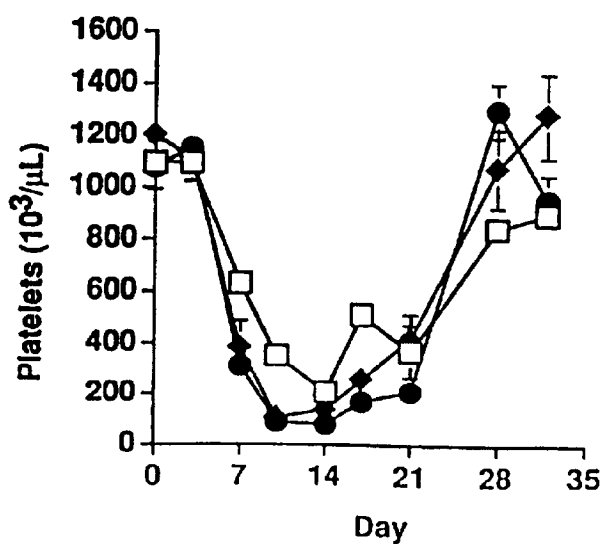


FIG. 15C



**FIG. 16A**

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scrFI      nlaIII      styI      ncoI      maeII      rsaI      csp6I      bsrI      nlaIII      acII      acII      hqal      maeIII      acII      pleI      hinfI      rsaI      csp6I      rsaI      csp6I      mnlI      rsaI      csp6I      mnlI
mvaI      dsai      hphI      acII
ecorII     sau96I      bstNI      haeIII/palI      asuI      apyI(dcm+)      bsrI      nlaIII      rsaI      csp6I      rsaI      csp6I      mnlI      rsaI      csp6I      mnlI
acII       bgII      dsav      sau96I      bstNI      haeIII/palI      asuI      apyI(dcm+)      bsrI      nlaIII      rsaI      csp6I      rsaI      csp6I      mnlI      rsaI      csp6I      mnlI
301 AAATGCGCG CCTGGCATT TACCCAGTAC ATGACCTTAT GGGACITTC TACTTGGCAG TACATCTACG TATTAGTCAT CGCTATTACC ATGGTGATGC
TTTACCGGCG GGACCGTAAT ACGGGTCATG TACTGGATA CCTGGAAGG ATGAACCGTC ATGTAGATCC ATATCAGTA CGGTATATGG TACCACTACG

401 CGTTTGGCA GTACATCAAT GGGCGTGGAT AGCGGTTTGA CTCACGGGA TTTCCAAGTC TCCACCCCAT TGACGTCAAT GGGAGTTTGT TTTGGCACCA
CCAAAACCGT CATGTAGTTA CCGGCACCTA TCGCCAAACT GAGTGCCCT AAGGTTTCA AGTGCGGTA ACTCCAGTTA CCTCAACA AAACCGTGGT

501 AAATCAACGG GACTTCCAA ATGTGCTAA CAACTCCGCC CCATTGACCC AAATGGCGG TAGGCGTGA CGGTGGGAGG TCTATATAAG CAGAGCTCGT
TTAGTTGCC CTGAAAGTT TTACAGCATT GTTGAGCGCG GGTAACTCGG GTTACCTCGG TTTACCGGCC ATCCGCACAT GCCACCTCC AGATATATTC GTCTGAGCA

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FIG. 16B

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haeIII/palI
mcrI
eagI/xmaIII/ecI XI
eaeI
cfrI
fnu4HI
aciI
thaI
fnuDII/mvnI
fnuDII/estII
sacII/estII
nspBII
kspI scrFI
dsal nciI
bglI bslI mspI
sau3AI mnlI bstUI
mboI/ndeII[dam-] hpaII
dpsI[dam+] bsaJI dsav
dpsII[dam-] bsh1236I
alwI[dam-] aciI cauII
601 TTAGTGAACC GTCAGATCGC CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGACCC GATCCAGCCT CCGCGGCGCG GAAAGGTGCA
AATCACTTGG CAGTCTAGCG GACCTCTGCG GTAGGTGCGA CAAACTGGA GGATCTTCT GTGGCCCTGG CTAGGTGCGA GGCGCGCGCC CTGGCCACGT
~Begin RNA

tfII
aciI
thaI hinfI
fnuDII/mvnI
bstUI
bsh1236I
701 TTGGAACCGG GATTCGCCGT GCCAGACGTG ACCTAAGTAC CGCCTATAGA GTCTATAGCC CCACCCCTTT GGCTTCGTGA GAAAGCGGCT ACAATTAATA
AACCTTGCGC CTAAGGGGCA CGGTTCTCAC TGCATTCTAC GCGGATATCT CACATATCCG GGTGGCGGAA CCGAAGCAAT CTGGCGCGCA TGTTAATTAT
~sp6 promoter

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FIG. 16C

[illegible]

FIG. 16D

[illegible]

FIG. 16E

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scrFI      eam11051
mval       sau961
ecorII
dsav
bstNI
bsai
bsaJI      hphI      maeII
           ddel apyI[dcM+]
           mnlI bsaJI acII bstEII
           1401 GACCTCAGCC CTGGGTGCGG GGTACCGAC AAAACTCACA CATGCCACC GTGCCACCA CTGAACTCC TGGGGGACC GTGAGTCTTC CTCTTCCCC
           CTGGAGTCGG GACCCACGCC CCAGTGGCTG TTTTGAGTGT GTACGGGTGG CACGGGTCTT GGACTGAGG ACCCCCTGG CAGTCAGAG GAGAGGGGG
           162 AspLeuSerp roglyCysGI yValThrAsp LysThrHist hrCysProPr oCysProAla ProGluLeuL euGlyGlyPr oSerValPhe LeuPheProPro
           ^insertion of a gly
           ^START OF HUMAN I9G1 CH2CH3

           sau96I
           nlalV
           nspl
           hpall
           scrFI
           nclI
           dsav
           sau3AI      avall
           mboI/ndelI[dam-]      nlalII
           nlalII      caulI      mnlI      nspl
           rcal dplI[dam+]      ddel      nspl
           bspHI[dam-]      asuI      eco8II      maeII
           styI      mnlI      dplII[dam-]      bsu36I/matII/sauI
           bsaJI      1501 CAAACCCCA GGACACCTC ATGATCTCCC GGACCCCTGA GGTCACATCC GTGGTGGTGG ACGTGAGCCA CGAGACCCCT GAGGTCAAGT TCMACTGGTA
           GTTTGGGTT CCTGTGGGAG TACTAGAGG CCTGGGACT CCAGTGATCG CACCACCACC TGCACCTGGT GCTTCTGGA CTCACGTTCA AGTTGACCAT
           196 LysProLy sasPthrLeu MetileSera rgtThrProGI uValThrCys ValValVala spValSerHI sGluAspPro GluValLysP heAsntrpTyr

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FIG. 16F

**FIG. 16G**

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mspI      dsal
hpaII     hphI
fnu4HI    mnlI
bbvI      nlaIV mboII scfI aluI bsaJI
1901 CGTGGAGTGG GAGAGCAATG GGACGCCGA GAACAACCTAC AAGACCACGC CTCGGTGCT GACTCCGAC GGCTCCTTCT TCCTCTACAG CAAGCTCACC
GCACCTCACC CTCTGGTTAC CCGTCGGCCT CTGTGTTGATG TTCTGGTGG GAGGCACGA CCGAGGCTG CCGAGCAAGA AGCAGATGTC GTTCGAGTGG
329 ValGluTrp GluSerAsnG lyGlnProG lYsnAsnTyr LysThrThrP roProValLe uAspSerAsp GlySerPheP heLeuTyrSe rLysLeuThr
pleI      hinfI
nlaIII    mnlI
ppu10I    nlaIII
nsII/avaII
2001 GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC TCCTGTGCTC
CACCTGTTCT CCGCCACCGT CGTCCCTTG CAGAAGAGTA CGAGCACTA CGTACTCCGA GACGTGTTGG TCATGTGGGT CTCTCGGAG AGGACACAGAG
362 ValaspLysS eArgTrpG l nGlnGlyAsn ValPheSerC ySerValMe tHisGluAla LeuHisAsnH lTyrThrGl nLysSerLeu SerLeuSerPro
mbolI     nlaIII
bpuAI     ppulOI
maeII     nsII/avaII
sfanI     mnlI
fnu4HI    xanI bbsI
bbvI      asp700
bapMI     fnu4HI
bbvI      xanI bbsI
2001 GTGGACAAGA GCAGGTGGCA GCAGGGGAAC GTCTTCTCAT GCTCGTGAT GCATGAGGCT CTGCACAACC ACTACACGCA GAAGAGCCTC TCCTGTGCTC
CACCTGTTCT CCGCCACCGT CGTCCCTTG CAGAAGAGTA CGAGCACTA CGTACTCCGA GACGTGTTGG TCATGTGGGT CTCTCGGAG AGGACACAGAG
362 ValaspLysS eArgTrpG l nGlnGlyAsn ValPheSerC ySerValMe tHisGluAla LeuHisAsnH lTyrThrGl nLysSerLeu SerLeuSerPro
sau96I    nlaIII
pleI      scfI
rmalI     hincII/hindII
sau96I    hinfI pstI
haeIII/paI
asul      maeI accI bspMI hindIII hinfI bspMI hindIII acil asul
2101 CCGGTAATG AGTGGACGG CCTAGAGTC GACCTGCAGA AGCTTCTAGA GTCGACCTGC AGAAGCTTGG CCGCCATGGC CCAACTTGT TATTGCAGCT
GCCCATTTAC TCACGCTGCC GGCATCTCAG CTGGACGTCT TCGAAGATCT CAGCTGGACG TCTTGAACCG GCGGTACCG GGTGCAACAA ATAACGTGCA
396 GlyLys
sau96I    nlaIII
fnu4HI    haeIII/paI
bgli      styI
sfII      ncoI
eaeI      dsal
cfri      bsaJI
aluI      haeIII/paI
fnu4HI    aluI
bbvI      fnu4HI
bbvI      bbvI
^sv40     early poly A
maeIII    sfanI apol
bsmI      maeI
rmalI
2201 TATTAATGGT ACAATAAAG CAAATAGCAT ACAAAATTCA CAAATAAAGC ATTTTTC A CTGCATTCTA GTTGTGGTTT GTCCAAACTC ATCAATGTAT
ATATTACCAA TGTTATTTC GTTATCTAG TGTTAAAGT GTTTATTTCG TAAAAAAGT GACGTAAGAT CAACACCNAA CAGGTTTCAG TAGTACATA

```

FIG. 16H



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[illegible]

FIG. 16I

[illegible]

**FIG. 16J**

**FIG. 16K**

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**FIG. 16L**

**FIG. 16M**

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```

scrFI
ncil
mspl
hpall
dsav
cauli
bsli
xmal/pspAI
smai
scrFI
ncil
dsav
cauli
bsaJI
bsaJI
bsli aval
3801 AAGGTTGGG CTTCGAATC GTTTCCGGG CAAAGGCCG CTAAGGAGG GTCCGGCCG
TTTCCAAACC GAAGCTTAG CAAAGGCCG CTAAGGAGG GTCCGGCCG
ATCTATGCT GCAGTTCCTC GCCCACCCTG GGAGATGGGG
HSV1 tk terminator SmaI-PvuII
hinPI
hhal/cfoI
thai
fnuDII/mvnl
bstUI
bsli
bsaJI
nlaIV acil
3901 GAGGCTAACT GAAACACGGA AGGAGACAAT ACCGGAAGCA ACCCGGCTA TGACGGCAAT AATAAAGCAG AATAAAGCAG ACAGGTGTG GTCGTTTGT
CTCCGATTGA CTTTGTGCTT TCCCTGTGTTA TGGCCTTCTT TGGGCGCGAT ACTGCGGTTA TTTTCTGTC TTATTTTGGC TGCCCAACAC CCAGCAACAA
scrFI
mval
ecorII
dsav
bstHI
bsaJI
bsli
sau96I
nlaIV
fnuDII/mvnl
bsaJI
bstUI
bsli236I
acil
thai
3801 AAGGTTGGG CTTCGAATC GTTTCCGGG CAAAGGCCG CTAAGGAGG GTCCGGCCG
TTTCCAAACC GAAGCTTAG CAAAGGCCG CTAAGGAGG GTCCGGCCG
ATCTATGCT GCAGTTCCTC GCCCACCCTG GGAGATGGGG
HSV1 tk terminator SmaI-PvuII
hinPI
hhal/cfoI
thai
fnuDII/mvnl
bstUI
bsli
bsaJI
nlaIV acil
3901 GAGGCTAACT GAAACACGGA AGGAGACAAT ACCGGAAGCA ACCCGGCTA TGACGGCAAT AATAAAGCAG AATAAAGCAG ACAGGTGTG GTCGTTTGT
CTCCGATTGA CTTTGTGCTT TCCCTGTGTTA TGGCCTTCTT TGGGCGCGAT ACTGCGGTTA TTTTCTGTC TTATTTTGGC TGCCCAACAC CCAGCAACAA
scrFI
mval
ecorII
dsav
bstHI
bsaJI
bsli
sau96I
nlaIV
fnuDII/mvnl
bsaJI
bstUI
bsli236I
acil
thai
4001 TCATAAACGC GGGGTTCCGT CCCAGGGCTG GCACCTCTCTC GATACCCCAAC CCAGACCCCA TTGGGGCCAA TAGCCCGCGG TTTCTTCTT TTCCCAACCC
AGTATTGGC CCCCACCCCA GGCTCCCGAC CGTGAGACAG CTATGGGGTG GCTCTGGGTG AACCCCGGTT ATCCCGCGG ATCCCGCGG AACAGAGAA AAGGGTGGG

```

FIG. 16N

4101 CAACCCCAAG GTTCGGGTG AGCCCAAGG CTCGACGCCA AGTCGGGGC GGCAGCCCG ACCTGCGGC CCGTCCGGC GGTATCGGT CCGGGGGC CCAATCCCTG CCCAGGGGG  
 GTTGGGGGT CAGCCCACT TCGGGTCC GAGGTCTGGT TCGAGCCCG CCGTCCGGC GGTATCGGT CCGGGGGC CCAATCCCTG CCCAGGGGG  
 4201 ATGGGAATG GTTTATGTT CCGTGGGGT ATTCTTTTG GCGTTGGTG GGTTCAGTC CACGACTGGA CTGAGCAGAC AGACCCATGG TTTTGGATG  
 TACCCCTTAC CAATACCA GAACCCCA TAAGAAACC CGCAAGCAC CCAGTCCAG GTGCTACCT GACTGCTG TCTGGGTACC AAAACCTAC  
 4301 GCTTGGGCAT GGACCGCATG TACTGGCGG ACAGCAACAC CGGGCGTCTG TGGCTGCCA ACACCCCGA CCCCCAAAA CCACCGCGG GATTCTGGC  
 CGGACCCGTA CCGGGCTAC ATGACCGGC TGTCTTGTG GCGCGCAGAC ACCGACGTT TGTGGGGCT GGGGTTTTT GTGGCGCG CTAAGACCG

FIG. 160

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```

acII      acII
thAI      thAI
fnuDII/mvnl  fnuDII/mvnl
bstUI     bstUI
          sacII/sstII
          haelIII/palI bsh1236I
          mcrI      nspBII
          dsal      kspI
          bsaJI     dsal
          hphI eagI/xmaIII/ecI XI
          maeIII eaeI bsaJI
          bstEII cfrI acII
          TTTGTATTGG TCACCACGGC CGAGTTTCCG
          AACATAACC AGTGGTGCCG GCTCAAGGCG

mspI      mspI
hpaII     hpaII
acII      acII
fnu4HI    fnu4HI
          bslI      sfaNI
          GAACTAAACC TGACTACGGC ATCTCTGCC CTTCTTCGCT
          CTTGATTGG ACTGATGCCG TAGAGACGGG GAAGAAGCGA CCATGCTCTCT CGCGAACAACA
          mboII     mboII
          csp6I    csp6I
          rsaI     rsaI
          mnlI     mnlI
          haeII    haeII
          hhaI/cfoI hhaI/cfoI
          hinPI    hinPI
          bsaJI     bsaJI
          hphI eagI/xmaIII/ecI XI
          maeIII eaeI bsaJI
          bstEII cfrI acII
          TTTGTATTGG TCACCACGGC CGAGTTTCCG
          AACATAACC AGTGGTGCCG GCTCAAGGCG

scrFI     nlaIV
ncII      hgiCI
dsav      scrFI
cauII     mvaI
bsII      ecorII
bsII      dsav
bsaJI     bstNI
sau96I    bsaJI
nlaIV     haeIII/palI
avaII     eaeI
asui      cfrI bsp1286
ppuMI     mspI apyIIdcm+)
nlaIV     hpaII bmyI
eco109I/draII banI
          nlalII
          bbsI
          mboII
          bpuAI
          fnu4HI
          acII     bcoGI
          GCGGCGACGA TAGTCATGCC CGCGGCCAC CGGAAGGAGC
          CCGTCTGCT ATCAGTACGG GCGCGGGTG GCCTTCTCTG
          *pBR322 sequence

4401
4501

```

FIG. 16P



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```

fnu4HI          acII
haeIII/palI    fnu4HI
mcrI           thal
eagI/xmaIII/ecI XI  fnuDII/mvni
eaeI           bstUI
notI           hinPI
fnu4HI         bstUI scfI
aciI           bsh1236I
mcrI bsrBI aciI    rsaI hhaI/cfoI fnu4HI tru9I aciI
sfanI taqI cfrI sfanI    csp6I bali aciI mseI bsh1236I
4601 TCACTGGGTT GAAGCTCTC AAGGCGATCG GTCAGCGGC CGCATCAAG CAACCATAGT ACGCGCCTG TAGCGGCGCA TTAGCGCGG CGGCTGTGTT
ACTGACCCAA CTTCCGAGAG TTCCGTAGC CAGCTCGCG CGGTAGTTC GTTGATATCA TGCGCGGAC ATCGCGCGCT AATTCGCGCC GCCACACCA
      bari
      delta 3
      *M13 ori

fnu4HI          hinPI
hinPI          hhaI/cfoI
thal           fnuDII/mvni
fnu4HI         bstUI
bstUI          bsh1236I
maeIII bbvI maeIII aciI
4701 GGTACGGC AGGTGACCG CTACACTGC CAGCGCCTA GCGCGCGCTC CTTTCGCTTT CTTCCTTCC TTTCGCGCA CGTTGCGCGG CTTTCCCCGT
CCATGCGCG TCGCACTGCG GATGTGACG GTCGCGGAT CCGCGCGGAG GAAGCGGAG GAAGAGCGGT GCAAGCGGCT GAAAGCGGCA

nlaIV          hgiJII
hgiJII         bsp1286
bmyI           banII
aluI           nlaIV
4801 CAAGCTCTAA ATCGGGGCT CCTTTAGG TTCCGATTTA GTGCTTTACG GCACCTGAC CCCAAAAAC TTGATTGGG TGATGGTTCA CGTAGTGGG
GTTGAGATT TAGCCCCGA GGAATCCC AAGCTAAT CAGGAATGC CGTGGAGCTG GGGTTTTTC AACTAAACCC ACTACCAAGT GCATCACCCG

maeIII pleI    tru9I pleI
drdI hinfi maeIII maeI hinfi
4901 CATGCCCTG ATAGACGGTT TTTCGCCCTT TGAGGTGGA GTCCACGCTC TTTAATAGT GACTCTGTT CCAACTGGA ACAACACTCA ACCCTATCTC
GTAGCGGAC TATCTGCTAA AAGCGGGA ACTGCAACCT CAGGTGCAAG AAATTATCAC CTGAGAACA GGTTGACCT TGTGTGAGT TGGATAGAG

```

FIG. 16Q

**FIG. 16R**

scrl  
 ncil  
 mspl  
 hpall  
 dsav  
 caull  
 hinll/acyl  
 hgal  
 ahalI/bsal  
 bcgl  
 mcrI fnu4HI  
 acil  
 fnuDII/mvnl  
 bstUI  
 bshl236I  
 hinPI  
 hhal/cfol  
 GTGGCGGCT ATTATCCCGT GATGACGCCG GGCAAGACGA ACTCGTGGC CGCATACACT  
 CAATGATGAG CACTTTTAA GTTCTGCTAT  
 CTTCCAAAG GTTACTACTC GTGAAATTT CAAGACGATA CACCGGCCA TAATAGGCA CTACTGGCG CCGTTCCTGT TGAGCCAGCG CGGTATGTGA  
 5501  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 5701  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 5801  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 5901  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 6001  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 6101  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 6201  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 6301  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 6401  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 6501  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 6601  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 6701  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 6801  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 6901  
 maelI  
 psp1406I  
 xmi  
 bmyI  
 hglAI/aspHI  
 bsp1286 tru9I  
 bsiHRAI msel  
 ahalII/dral  
 GTTCTGCTAT  
 CTTCTGCTAT  
 CAAGATGAGT  
 TACTTCTGAC  
 AACGATCGGA  
 GGACCGNAGG  
 AGCTAACCGC  
 TTTTTCAC  
 AACATGGGG  
 ATCATGTAAC  
 TCGCCTTGAT  
 ACTATTGTGA  
 CGCCGGTTGA  
 ATGAAGACTG  
 TTGCTAGCCT  
 CCTGGCTCC  
 TCGATTGGCG  
 AAAAAACGTG  
 TTGTACCCC  
 TAGTACNTG  
 AGCGGAACTA  
 7001

FIG. 16S

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```

mspi          bglI
hpall         sau96I
scrFI         haeIII/pall
              haeIII/pall
              hinpI asuI mspi
              hhaI/cfoI hpall
5901 AACTACTTAC TCTAGCTTCC CGGCACCAAT TAATAGACTG GATGAGCGG GATAAGTTG CAGGACCACT TCTGGCTCG GCCCTTCCGG CTGGCTGGTT
      TTGATGAATG AGATCGAAGG GCCGTGTTA ATTATCTGAC CTACCTCGC CTATTCAAC GTCTGTGA AGACCGAGC CGGGAAGGCC GACCGACCAA

              aciI
              thai
              fnuDII/mvni sau96I
              bstUI asuI
              bsmAI fnu4HI nlaIV mnlI
              bsal bsh1236I bbvI bsrI haeIII/pall
6001 TATTGCTGAT AAATCTGGAG CCGGTGAGCG TGGTCTCGG GGTATCATTG CAGCACTGG GCCAGATGGT AAGCCCTCCC GTATCGTAGT TATCTACACG
      ATAACCACTA TTAGACCTC GCCCACTCGC ACCCAGAGCG CCATAGTAAC GTCTGACCC CGTCTACCA TTCGGAGGG CATAGCATCA ATAGATGTC

              ddeI
              sau3AI nlaIV
              mboI/ndeII[dam-] mnlI
              dpnI[dam+] hgiCI tru9I
              dpnII[dam-] bani msel maelII
              foki
              pleI
              hinfi
6101 ACGGGGAGTC AGGCAACTAT GGATGAACGA AATAGACAGA TCGCTGAGT AGTGCCCTCA CTGATTAAAGC ATTGTAACG GTGAGACCAA GTTACTCAT
      TCGCCCTCAG TCGCTGATA CCTACTGCT TTATCTGTCT AGCGACTCTA TCCACGGAGT GACTAATTCG TAACCATGTA CAGTCTGGTT CAAATGAGTA

              hphI
              rmaI sau3AI
              sau3AI mboI/ndeII[dam-]
              mboI/ndeII[dam-]
              dpnI[dam+] dpnI[dam+]
              tru9I dpnII[dam-] dpnII[dam+]
              ahaIII/draI mael alwI[dam+]
              tru9I bstYI/xhoII bstYI/xhoII nlaIII
              msel msel alwI[dam-] mboII[dam-] rcaI
              ahaIII/draI msel msel alwI[dam-] mboII[dam-] bspHI
6201 ATATACITTA GATTGATTAA AACTTCATT TTAAATTAA AAGGATCTAG GTGAAGATCC TTTTGATAA TCTCATGACC AAATCCCTT AACGTGAGTT
      TATATGAAT CTAACTAAT TTGAAGTAA AATTAAATT TTCTAGATC CACTCTAGG AAAACTATT AGAGTACTGG TTTAGGAA TTGCACTCA

```

FIG. 16T

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```

sau3AI
mbol/ndeII(dam-)
dpmI(dam+) sau3AI
dpmI(dam-) mbol/ndeII(dam-)
bstYI/xhoII dpmI(dam+) fnuDII/mvnI
sau3AI alwI(dam-) dpmI(dam-) bstUI
mbol/ndeII(dam-) alwI(dam-) bsh1236I
dpmI(dam+) mbolI(dam-) hinPI fnu4HI
dpmI(dam-) bstYI/xhoII hhaI/cfoI bbvI
6301 TTGTTTCCAC TGAGCGTCAG ACCCGTAGA AAGATCAAA GGATCTCTT TTTCTGCGC GTATCTGCT GCTTGCACAC AAAAAACCA
AAGCAGGTG ACTGCAGTC TGGGCATCT TTTCTAGTTT CTTAGAGAA CTCTAGCAA AAGACCGCG CATTAGACGA CGAACGTTG TTTTITGGT
dclI hgaI
sau3AI
mbol/ndeII(dam-)
dpmI(dam+)
dpmI(dam-)
alwI(dam-)
mspI
hpaII
alul
6401 CCGTACGAG CGGTGGTTG TTTCCGGAT CAAGAGCTAC CAATCTTTT TCCGAGGTA ACTGGCTTCA GCAGAGCGCA GATACCAAT ACTGTCTTC
GGGATGTC GCCACCAAC AAGCGCTA GTTCTCGATG GTTGAGAAA AGGCTTCCAT TGACCGAAGT CGTCTCGCT CTATGGTTA TGACGGAG
acII nsp8II
bsII haeIII/palI haeI
6501 TAGGTAGCC GTAGTAGCC CACCACTTCA AGAATCTGT AGCAGCGCT ACATACCTCG CTCTGCTAAT CCTGTTACCA GTGGCTGCTG CCAGTGGCGA
ATCAGATCG CATCAATCG GTGCTGAGT TCTTGAGACA TCGTGGCGA TGATGGAGC GAGACGATTA GGACAATGTT CACCGACCAC GGTACCGCT
fnu4HI
alwNI bbvI
bsrI fnu4HI
maeII bbvI bsrI
6601 TAAGTCTGT CTTACCGGT TGGACTCAAG ACGATAGTTA CCGATAAG CCGAGCGCTC GCGCTGAACG GGGGTTGCT GCACACAGCC CAGCTTGGAG
ATTACGACA GAATGGCCA ACCTGAGTTC TGCTATCAAT GCGTATTC CCGCTGCGAG CCGACTTGC CCCCCAGCA CGTGTGTCG GTCGAACCTC
hglAI/aspHI
bsp1286
bsiHKAI
bmyI
apaLI/snoI
alw44I/snoI
alul

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FIG. 16U

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6701 CGACGACCT ACACCGAAT GAGATACCTA CAGCGTGAGC ATTGAGAAAG CGCCACGCTT CCGGAAAGGA GAAGGGCGA acil fnu4HI
    CTTGCTGGA TGTGGCTTGA CTCTATGGAT GTGCACTGCG TAACTCTTC GCGTGCGAA GGGCTTCCCT GTTCCGCTT GTCCATAGGC CATTGCGCGT
    hinPI hhaI/cfoI hpaII bsaBI
    mspI hpaII bsaBI fnu4HI
    scrFI mvaI ecorII mvaI dsav bstNI bsaJI bsaNI
    6801 GGGTGGGAC AGGAGAGCGC ACAGAGGAGC TTCCAGGGGG AAACGCTGG TATCTTTATA GTCTGTGCGG GTTTCGCCAC CTCTGACTTG AGCGTGGATT
    CCGAGCCTTG TCTCTGCGG TGTCTGCTG TGTCTGCTG TTTGGGACC ATAGAATAT CAGGACAGCC CAAAGCGGTG GAGACTGAAC TCCGAGCTAA
    hinPI mnlI hhaI/cfoI aluI apyI[dcmt+] apyI[dcmt+] mnlI dndI hgaI
    6901 TTTGTGATCC TCGTCAGGGG GCGGAGGCT ATGGAANAAC GCCAGCTGGC ACAGACAGTT TCCCGACTGG AAAGCGGGA acil hhaI/cfoI mspI
    AAACACTAGC AGCAGTCCCC CCGCTGCGA TACCTTTTG CCGTCGACCG TCGTGTCAG TCGTGTCAG AGGGCTGACC TTTCCGCTT CACTCGCTT GCCTTAATTA
    aluI pvuII nspBII
    tru9I mspI
    scrFI mvaI ecorII dsav
    7001 GTGAGTTACC TCACTCATTG GGCACCCGAG CTTTACACT TTATGCTTCC GGTCTGTATG TTGTGTGGAA TTGTGAGCGG ATACAAATTT CACACAGGAA
    CACTCAATGG AGTGAGTAAT CCGTGGGCTC CGAATGTGA ATACGAAGG CCGAGCATAC AACACACCTT AACACTGCGC TATTGTAA GGTGTGCTT
    mali maeIII hgiCI apyI[dcmt+] bsaI bsaJI mspI hpaII
    aluI nlaIII asp700
    7101 ACAGCTATGA CCATGATTAC GAATTAA
    TGTGATACT GGTACTAATG CTTAATT
    tru9I maeI
    aaeI/asmI/vspI
    xmiI
    >length: 7127

```

FIG. 16V

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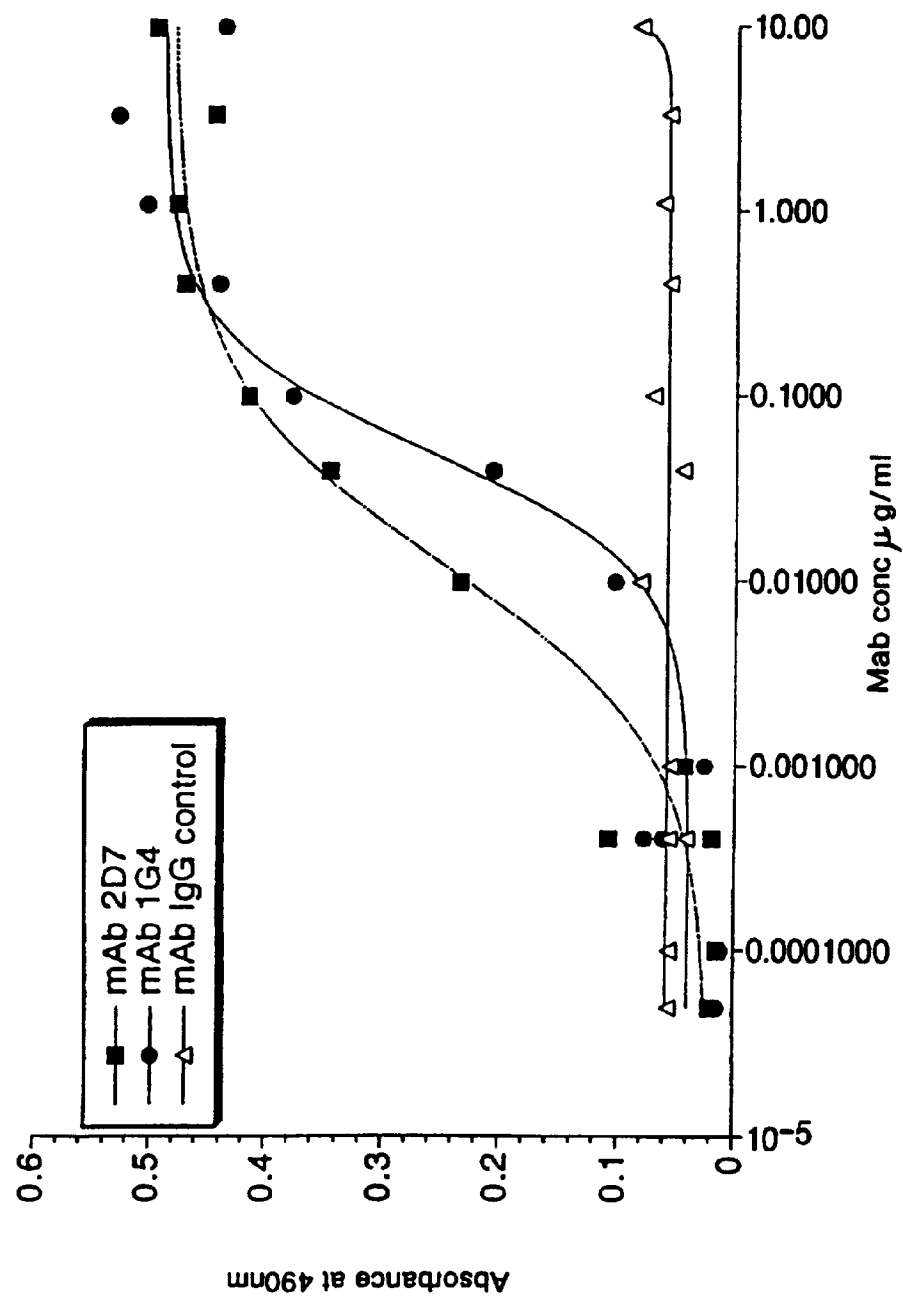


FIG. 17

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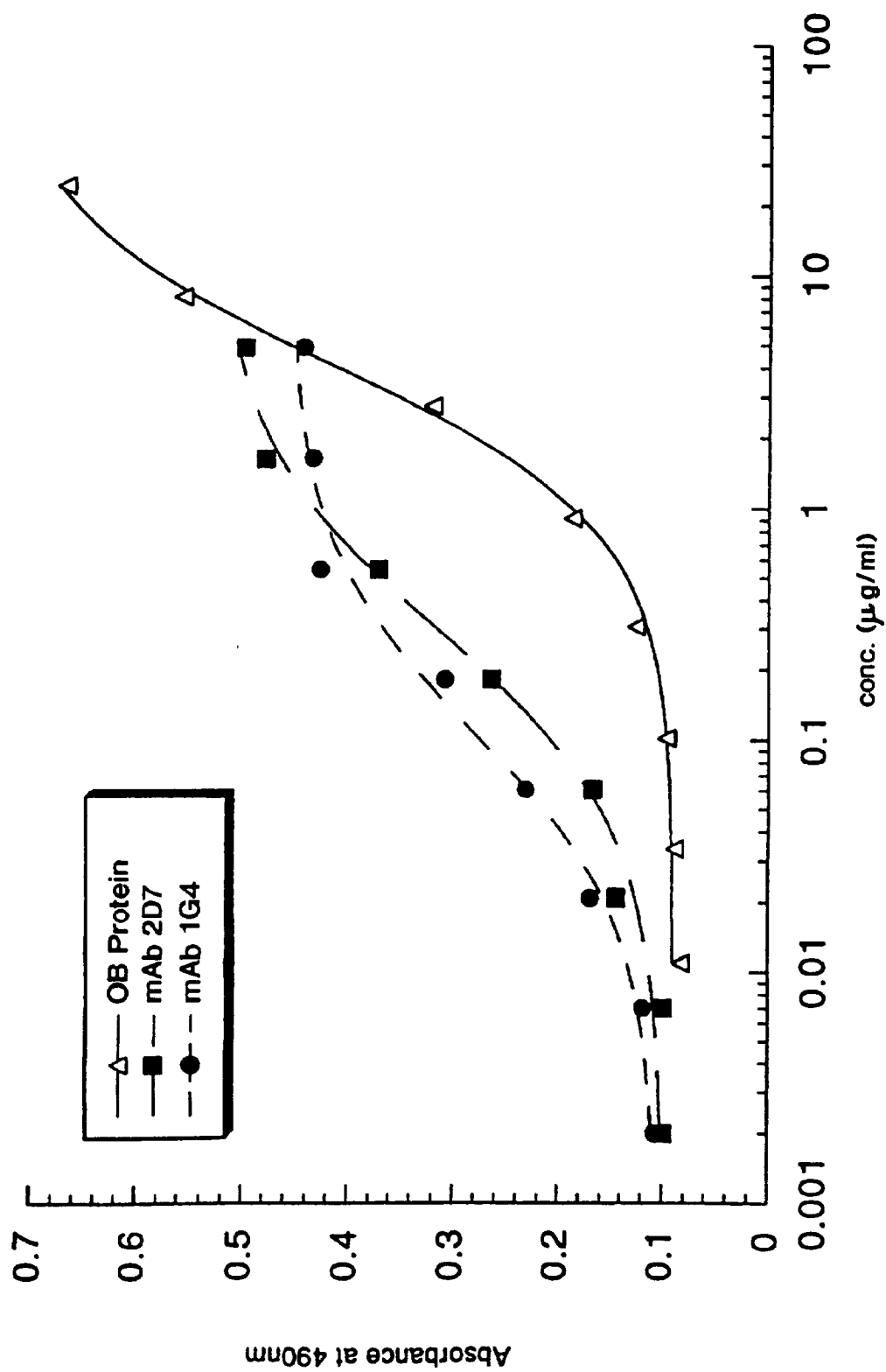


FIG. 18



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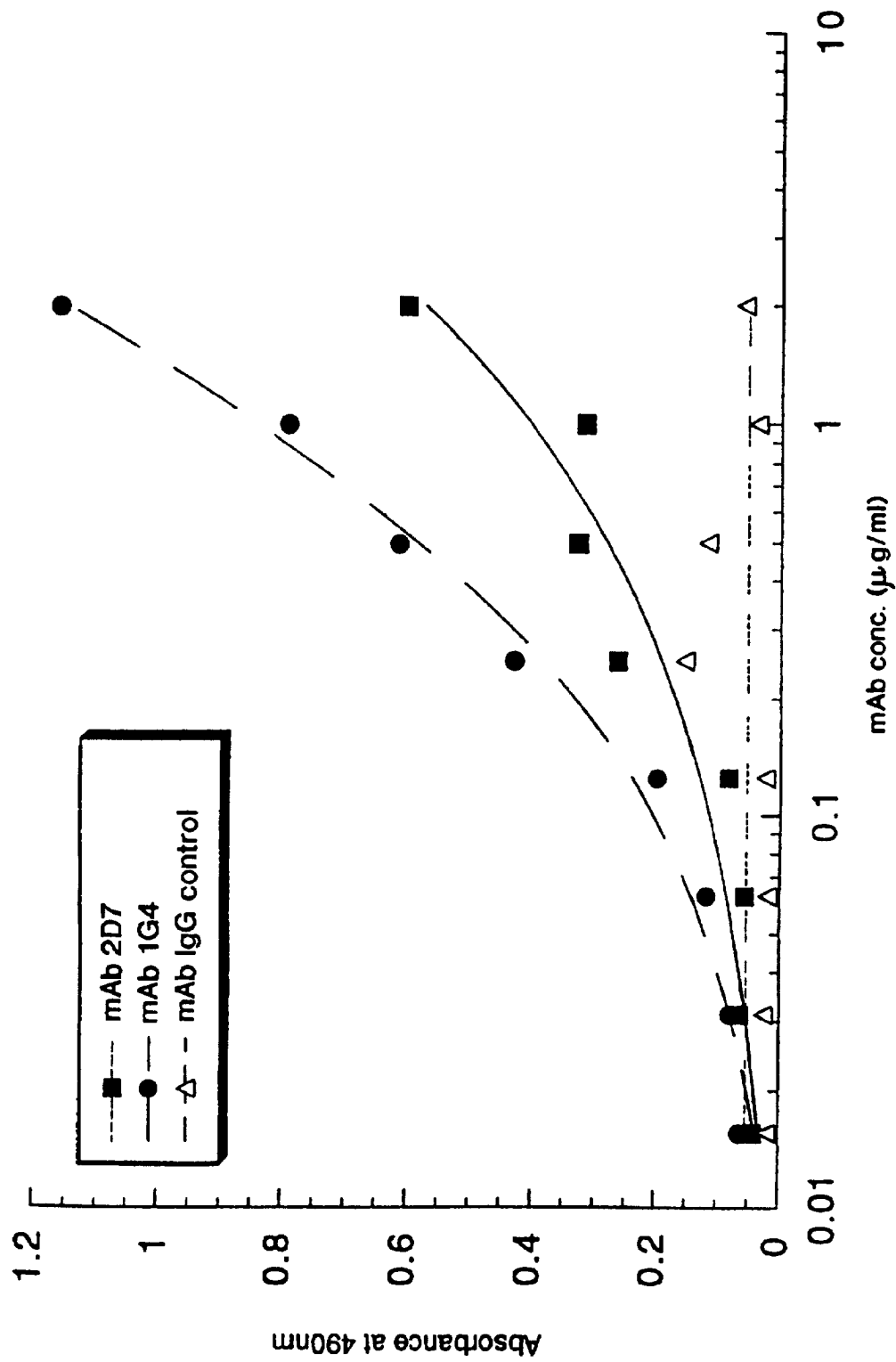


FIG. 19

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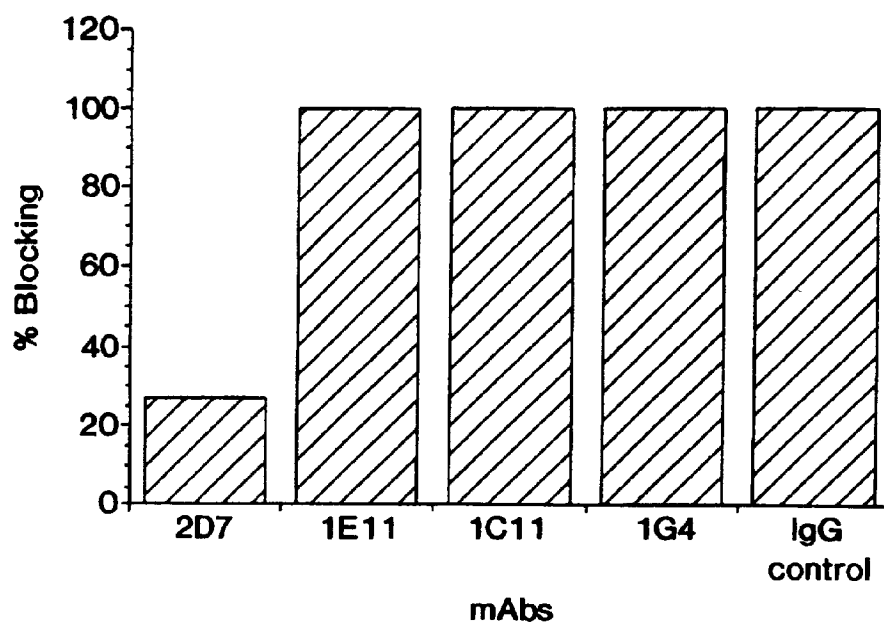


FIG. 20A

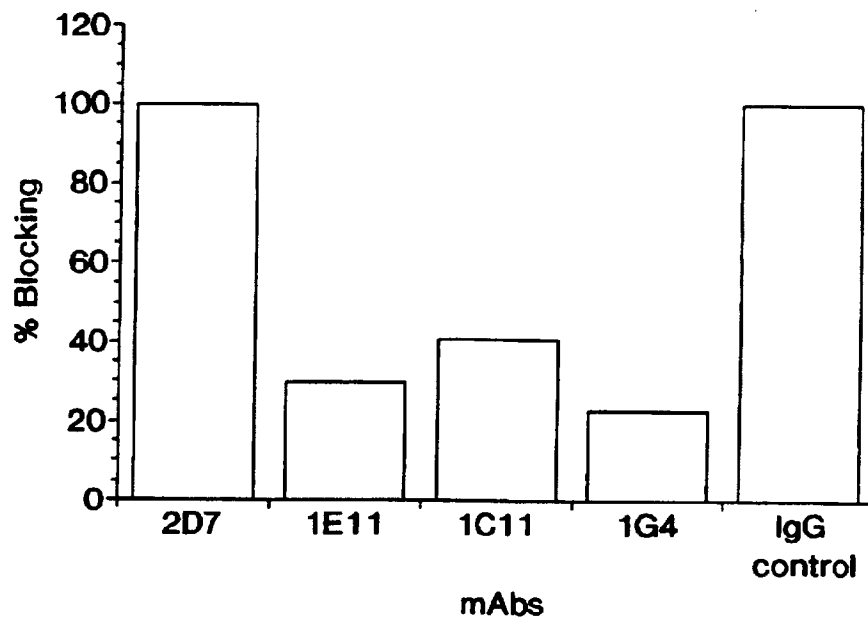


FIG. 20B

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1	M	I	C	O	K	F	C	V	V	L	L	H	W	E	F	I	Y	V	I	T	A	F	N	L	S	Y	P	I	T	P	W	R	F	K	L	S	C	M	P	P	N	S	T	Y	D	Y	F	L	L	P
1	M	M	C	O	K	F	Y	V	V	L	L	H	W	E	F	L	Y	V	I	A	A	L	N	L	A	Y	P	I	S	P	W	K	F	K	L	F	C	G	P	P	N	T	T	D	D	S	F	L	S	P
51	A	G	L	S	K	N	T	S	N	S	N	G	H	Y	E	T	A	V	E	P	K	F	N	S	S	G	T	H	F	S	N	L	S	K	T	T	F	H	C	C	F	R	S	E	Q	D	R	N	C	S
51	A	G	A	P	N	A	S	A	L	K	G	A	S	E	A	I	V	E	A	K	F	N	S	S	G	I	Y	V	P	E	L	S	K	T	V	F	H	C	C	F	G	N	E	Q	G	O	N	C	S	
101	L	C	A	D	N	I	E	G	K	T	F	V	S	T	V	N	S	L	V	F	Q	I	D	A	N	W	N	I	Q	C	W	L	K	G	D	L	K	L	F	I	C	Y	V	E	S	L	F	K	N	
101	A	L	T	D	N	T	E	G	K	T	L	A	S	V	V	K	A	S	V	F	R	Q	L	G	V	N	W	D	I	E	C	W	M	K	G	D	L	T	L	F	I	C	H	M	E	P	L	P	K	N
151	L	F	R	N	Y	N	Y	K	V	H	L	L	Y	V	L	P	E	V	L	E	D	S	P	L	V	P	Q	K	G	S	F	Q	M	V	H	C	N	C	S	V	H	E	C	C	E	C	L	V	P	V
151	P	F	K	N	Y	D	S	K	V	H	L	L	Y	D	L	P	E	V	I	D	D	S	P	L	P	P	L	K	D	S	F	Q	T	V	Q	C	N	C	S	L	R	G	-	C	E	C	H	V	P	V
201	P	T	A	K	L	N	D	T	L	L	M	C	L	K	I	T	S	G	G	V	I	F	Q	S	P	L	M	S	V	Q	P	I	N	M	V	K	P	D	P	P	L	G	L	H	M	E	I	T	D	D
200	P	R	A	K	L	N	Y	A	L	L	M	Y	L	E	I	T	S	A	G	V	S	F	Q	S	P	L	M	S	L	Q	P	M	L	V	V	K	P	D	P	P	L	G	L	H	M	E	V	T	D	D
251	G	N	L	K	I	S	W	S	S	P	P	L	V	P	F	P	L	Q	Y	Q	V	K	Y	S	E	N	S	T	T	V	I	R	E	A	D	K	I	V	S	A	T	S	L	L	V	D	S	I	L	P
250	G	N	L	K	I	S	W	D	S	Q	T	M	A	P	F	P	L	Q	Y	Q	V	K	Y	L	E	N	S	-	T	I	V	R	E	A	E	I	V	S	A	T	S	L	L	V	D	S	V	L	P	
301	G	S	S	Y	E	V	Q	V	R	G	K	R	L	D	G	P	G	I	W	S	D	W	S	T	P	R	V	F	T	T	Q	D	V	I	Y	F	P	P	K	I	L	T	S	V	G	S	N	V	S	F
299	G	S	S	Y	E	V	Q	V	R	S	K	R	L	D	G	S	G	V	W	S	D	W	S	S	P	Q	V	F	T	T	Q	D	V	V	Y	F	P	P	K	I	L	T	S	V	G	S	N	A	S	F

FIG. 21A

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351 H C I Y K K E N K I V P S K E I V W W M N L A E K I P Q S Q Y D V V S D H V S K V T F F I N L N E T K  
 349 H C I Y K N E N Q I I S S K Q I I V W W R N L A E K I P E I Q Y S I V S D R V S K V T F S N L K A T R

401 P R G K F T Y D A V Y C C N E H E C H H R Y A E L Y V I D V N I N I S C E T D G Y L T K M T C R W S  
 399 P R G K F T Y D A V Y C C N E Q A C H H R Y A E L Y V I D V N I N I S C E T D G Y L T K M T C R W S

451 T S T I Q S L A E S T L Q L R Y H R S S L Y C S D I P S I H P I S E P K D C Y L Q S D G F Y E C I F  
 449 P S T I Q S L V G S T V Q L R Y H R R S L Y C P D S P S I H P T S E P K N C V L Q R D G F Y E C V F

501 Q P I F L L S G Y T M W I R I N H S L G S L D S P P T C V L P D S V V K P L P P S S V K A E I T I N  
 499 Q P I F L L S G Y T M W I R I N H S L G S L D S P P T C V L P D S V V K P L P P S N V K A E I T V N

551 I G L L K I S W E K P V F P E N N L Q F O I R Y G L S G K E V Q W K M Y E V Y D A K S K S V S L P V  
 549 T G L L K V S W E K P V F P E N N L Q F O I R Y G L S G K E I Q W K T H E V F D A K S K S A S L L V

601 P D L C A V Y A V Q V R C K R L D G L G Y W S N W S N P A Y T V V M D I K V P M R G P E F W R I I N  
 599 S D L C A V Y V Q V R C R R L D G L G Y W S N W S S P A Y T L V M D V K V P M R G P E F W R K M D

651 G D T M K K E K N V T L L W K P L M K N D S L C S V Q R Y V I N H H T S C N G T W S E D V G N H T K  
 649 G D V T K K E R N V T L L W K P L T K N D S L C S V R R Y V V K H R T A H N G T W S E D V G N R T N

FIG. 21B

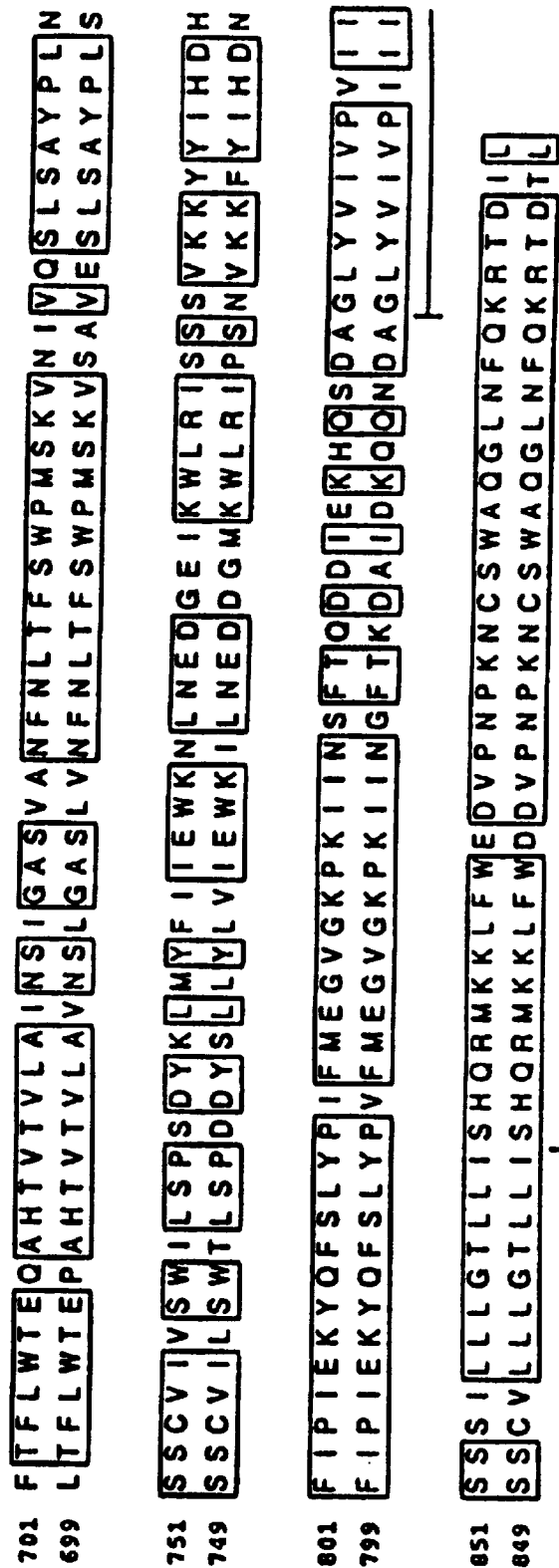
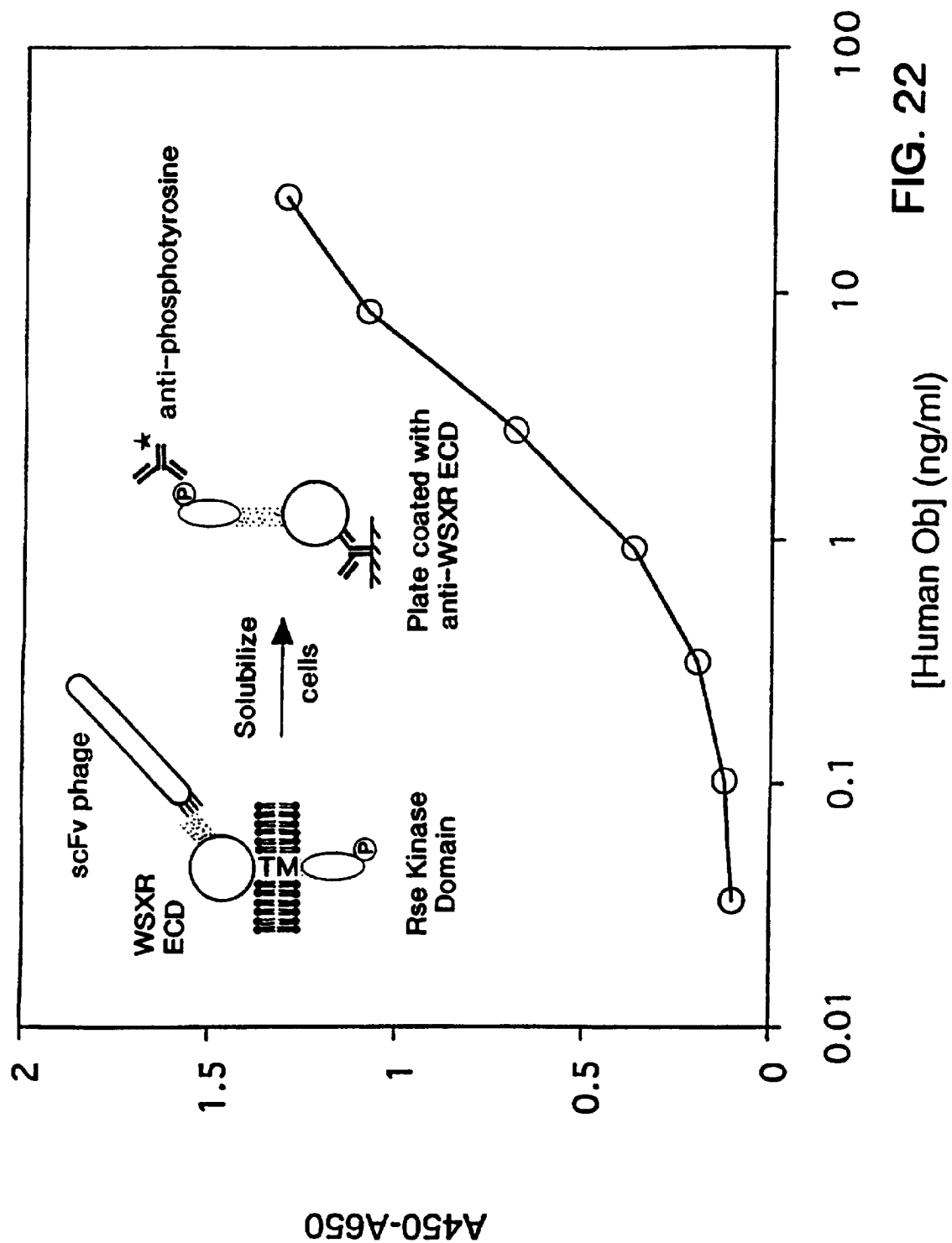


FIG. 21C



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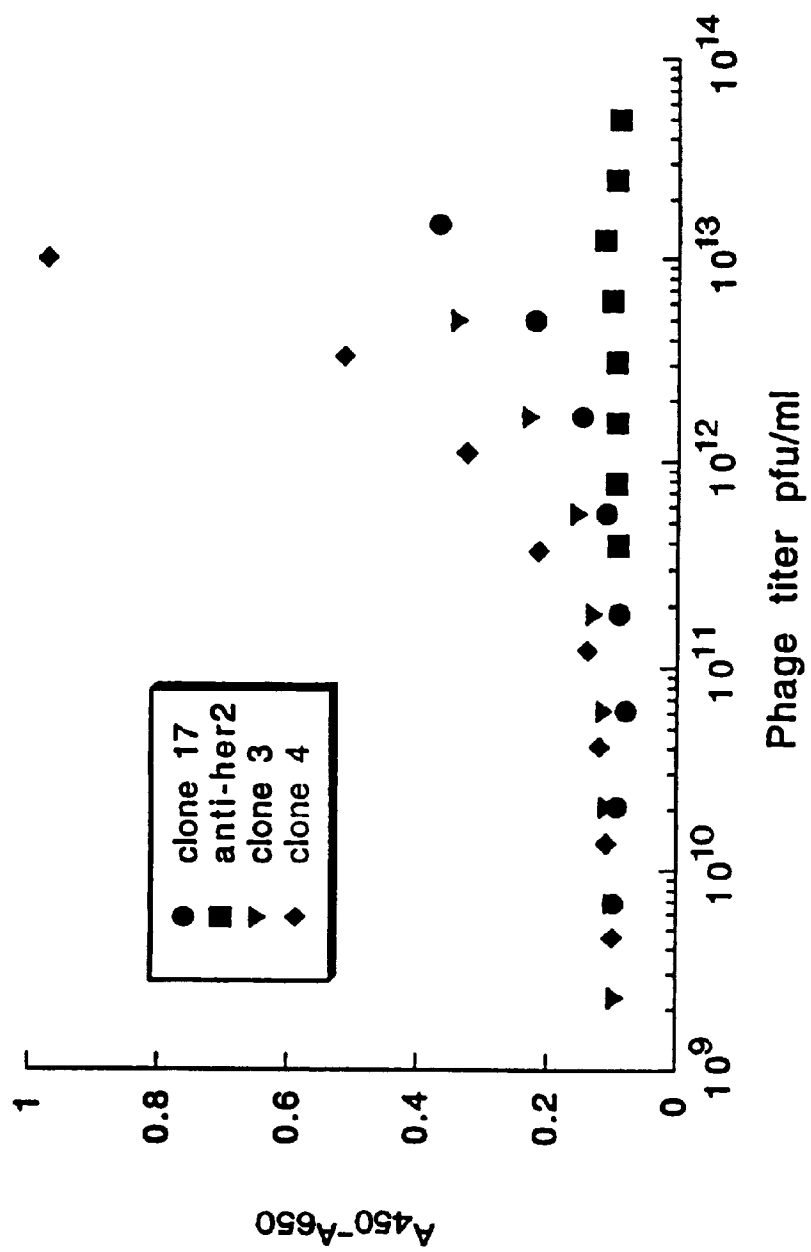


FIG. 23

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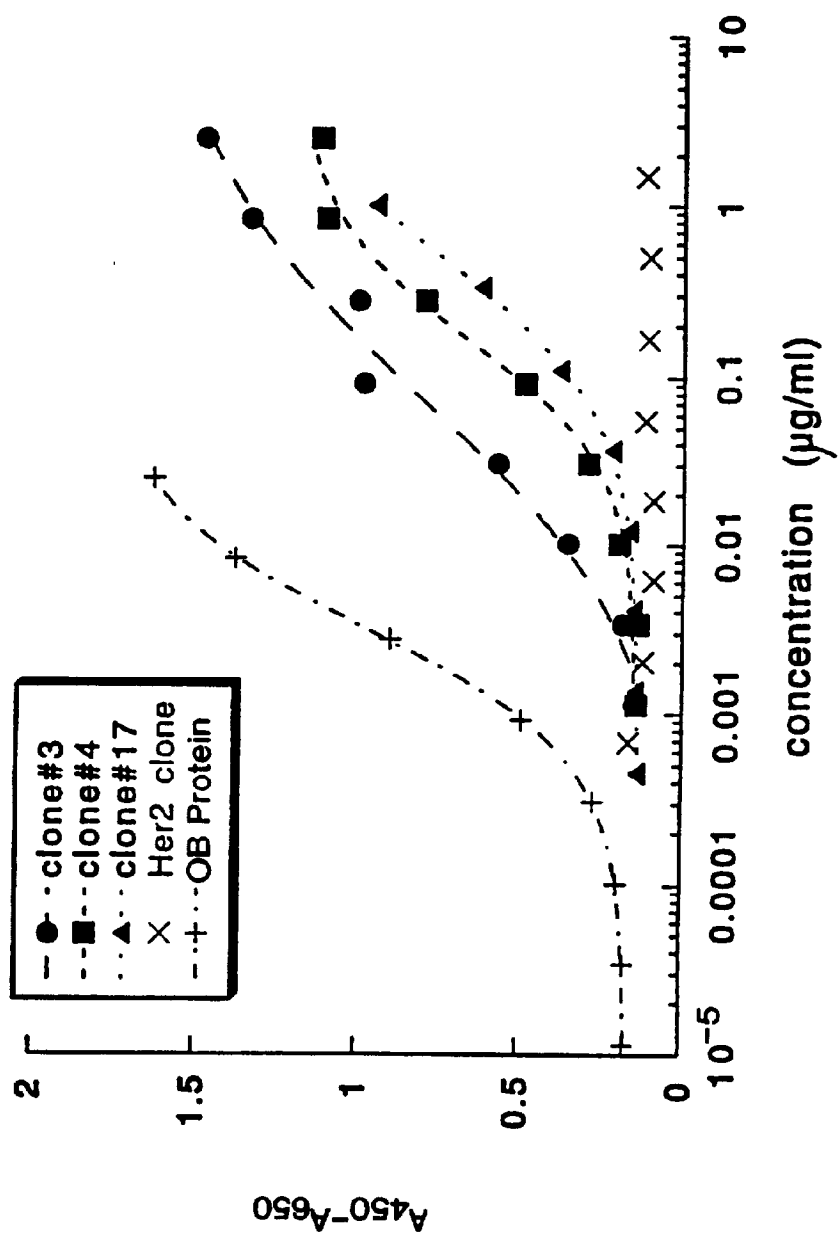


FIG. 24



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17.scfv 1 QVRLQQSGGGLVQPGRSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSG  
 3.scfv 1 EVQLVQSGAEVKKPGASVKVSCKASGYTFTGYMYWVRQAPGQGLEWMGW  
 4.scfv 1 EVQLVQSGAEVKKPGESLKISCQSGFTFSSYKMNWVRQAPGKGLEWMGG  
  
 CDR H1  
 17.scfv 51 MTWNSGSIGYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCAREP  
 3.scfv 51 INPNSGGTNYAOKFOGRVTMTTRDTSIGTAYMELSRLSSDDTAVYYCARDR  
 4.scfv 51 LIPIEGTANYAOKFOGRVTITADESTSTAYMELSSLRSED TAVYYCARDR  
  
 CDR H2  
 17.scfv 101 HNTDA-----EDIWGRGTLVTVSSGGGGPGGGGSGGGGSDVVMTQSP  
 3.scfv 101 YVGSSAYHRGSYYMDYWGRGTLVTVSSGGGGTGGGGSGGGGS-SELTQDP  
 4.scfv 101 VVVPATSLRGG--MDVWQGTTVTVSSGGGGSGGGGSGGGGSQSVLTQPA  
  
 CDR H3  
 17.scfv 143 SFLSAFVGDTITITCRASQ---GIYNYLAWYQQKPGKAPKLLIYAASTLO  
 3.scfv 150 A-VSVALGQTVRITCOGDS--LRSY-YASWYQQKPGQAPVLVIYGNRNP  
 4.scfv 149 S-VSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGKAPKLMIYEGSKRP  
  
 CDR L1 CDR L2  
 17.scfv 190 SGVPSRFSGSGSGTEFTLTISSLQPEDFGTYTCOOLI--SYPLTFGGGTK  
 3.scfv 196 SGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSRDSSGNHVVFGGGTK  
 4.scfv 198 SGVSNRFSGSKSGSTASLTISGLQAEDEADYYCSSYTTSTR-VFGGGTK  
  
 CDR L3  
 17.scfv 238 VEIK  
 3.scfv 246 LTVL  
 4.scfv 247 LTVL

FIG. 25

# INTERNATIONAL SEARCH REPORT

Intern: 1 Application No  
PCT/US 97/00325

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12N15/12 C07K14/715 C07K16/46 C07K19/00 C07K16/28  
C12N15/62 A61K39/395 C12N5/10 C12N15/85 G01N33/577  
G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CELL, vol. 83, no. 7, 29 December 1995, pages 1263-1271, XP000602068 TARTAGLIA L A ET AL: "IDENTIFICATION AND EXPRESSION CLONING OF A LEPTIN RECEPTOR, OB-R" cited in the application	1-4,7,8, 16-23, 25,26, 28-30, 32-37, 39,40, 46,47,58
Y	see the whole document	5,6, 9-15,31
Y	--- WO 94 05332 A (BERLEX LAB) 17 March 1994 see page 1 - page 10 see page 18; claim 16	5,6
Y	--- WO 91 01743 A (CEMU BIOTEKNIK AB) 21 February 1991 see page 1, line 1 - page 6, line 30 see page 18; claims	5
	--- -/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

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Date of the actual completion of the international search

21 April 1997

Date of mailing of the international search report

29. 04. 97

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Macchia, G

## INTERNATIONAL SEARCH REPORT

Intern: Application No

PCT/US 97/00325

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCE USA, vol. 88, 1991, pages 10535-10539, XP002029642 ASHKENAZI ET AL.: "Protection against endotoxic shock by a tumor necrosis factor receptor immunoadhesin" cited in the application see page 10535 ---	9-12,31
Y	US 5 378 808 A (D ANDREA ALAN ET AL) 3 January 1995 see column 3, line 5-19 see column 6, line 62 - column 7, line 34 ---	13-15
P,X	WO 96 08510 A (PROGENITOR INC) 21 March 1996 ---	1,2,4, 7-9,13, 14,16, 17, 19-23, 25,26, 28-36, 38,40, 46,47 37, 41-45, 48-57
A	see abstract  see page 1, line 16 - page 7, line 25 see page 9, line 1 - page 10, line 9 see page 16, line 8 - page 25, line 5 see page 50 - page 53; claims see figure 3B	
P,X	& NATURE MEDICINE, vol. 2, no. 5, 5 May 1996, pages 585-589, XP002019361 CIOFFI ET AL.: "Novel B219/0B receptor isoforms: possible role of leptin in hematopoiesis and reproduction" cited in the application	1,2,4,7, 8,16,23, 28-30, 32-35
A	see the whole document ---	25-27, 36,38-47
A	SCIENCE, vol. 271, 5 January 1996, page 29 XP002029643 BARINAGA M: "Obesity: Leptin receptor weighs in" see the whole document ---	13,14, 16,17,58
P,X	CURRENT BIOLOGY, vol. 6, no. 9, 1 September 1996, pages 1170-1180, XP000673008 BENNET ET AL.: "A role for Leptin and its cognate receptor in hematopoiesis" see the whole document -----	1-4,7-9, 16, 23-27, 29-39, 41-58

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern: Application No

PCT/US 97/00325

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9101743 A	21-02-91	AT 107514 T	15-07-94
		AU 652124 B	18-08-94
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		CA 2176463 A	21-03-96
		EP 0730606 A	11-09-96

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
26 April 2001 (26.04.2001)

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(10) International Publication Number  
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(51) International Patent Classification<sup>7</sup>: **C07K 14/00**

(74) Agents: **AGARWAL, Atulya, R.** et al.; Genentech, Inc., 1 DNA Way, South San Francisco, CA 94080-4990 (CA).

(21) International Application Number: PCT/US00/28827

(22) International Filing Date: 18 October 2000 (18.10.2000)

(25) Filing Language: English

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(30) Priority Data:  
60/160,542 20 October 1999 (20.10.1999) US

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(71) Applicant (*for all designated States except US*): **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **DE SAUVAGE, Frederic, J.** [BE/US]; 187 Shooting Star Isle, Foster City, CA 94404 (US). **GREWAL, Iqbal** [US/US]; 323 Turkshead Lane, Redwood City, CA 94065 (US). **GURNEY, Austin, L.** [US/US]; 1 Debbie Lane, Belmont, CA 94002 (US).

**Published:**

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WO 01/29070 A2

(54) Title: TYPE I CYTOKINE RECEPTOR TCCR

(57) Abstract: The present invention relates to methods for the treatment and diagnosis of immune related diseases, including those mediated by cytokines released primarily either Th1 or Th2 cells in response to antigenic stimulation. The present invention further relates to methods for biasing the differentiation of T-cells in either the Th1 subtype or the Th2 subtype, based on the relative expression levels of the gene TCCR, and its agonists or antagonists. The present invention further relates to a method of diagnosing Th1- and Th2-mediated diseases.

**TYPE I CYTOKINE RECEPTOR TCCR****Field of the Invention**

5           The present invention relates generally to the identification and isolation of novel DNA, the recombinant production of novel polypeptides, and to compositions and methods for the diagnosis and treatment of immune related diseases, specifically to methods of modulating the T-cell differentiation and cytokine release profiles into Th1 subtype and Th2 subtypes, and the host of disorders that are implicated by the release of the cytokine profiles.

**Background of the Invention**

10           Immune related and inflammatory diseases are the manifestation or consequence of fairly complex, often multiple interconnected biological pathways which in normal physiology are critical to respond to insult or injury, initiate repair from insult or injury, and mount innate and acquired defense against foreign organisms. Disease or pathology occurs when these normal physiological pathways cause additional insult or injury either as directly related to the intensity of the response, as a consequence of abnormal regulation or excessive stimulation, as a  
15           reaction to self, or as a combination of these.

          Though the genesis of these diseases often involves multistep pathways and often multiple different biological systems/pathways, intervention at critical points in one or more of these pathways can have an ameliorative or therapeutic effect. Therapeutic intervention can occur by either antagonism of a detrimental process/pathway or stimulation of a beneficial process/pathway.

20           T lymphocytes (T cells) are an important component of a mammalian immune response. T cells recognize antigens which are associated with a self-molecule encoded by genes within the major histocompatibility complex (MHC). The antigen may be displayed together with MHC molecules on the surface of antigen presenting cells, virus infected cells, cancer cells, grafts, *etc.* The T cell system eliminates these altered cells which pose a health threat to the host mammal. T cells include helper T cells and cytotoxic T cells. Helper T cells proliferate  
25           extensively following recognition of an antigen-MHC complex on an antigen presenting cell. Helper T cells also secrete a variety of cytokines, *i.e.* lymphokines, which play a central role in the activation of B cells, cytotoxic T cells and a variety of other cells which participate in the immune response.

          A central event in both humoral and cell mediated immune responses is the activation and clonal expansion of helper T cells. Helper T cell activation is initiated by the interaction of the T cell receptor (TCR) -  
30           CD3 complex with an antigen-MHC on the surface of an antigen presenting cell. This interaction mediates a cascade of biochemical events that induce the resting helper T cell to enter a cell cycle (the G0 to G1 transition) and results in the expression of a high affinity receptor for IL-2 and sometimes IL-4. The activated T cell progresses through the cycle proliferating and differentiating into memory cells or effector cells.

          The immune system of mammals consists of a number of unique cells that act in concert to defend the host  
35           from invading bacteria, viruses, toxins and other non-host substances. The cell type mainly responsible for the specificity of the immune system is called the lymphocyte, of which there are two types, B and T cells. T cells take their designation from being developed in the thymus, while B cells develop in the bone marrow. The T-cell population has several subsets, such as suppressor T cells, cytotoxic T cells and T helper cells. The T-helper cell

subsets define 2 pathways of immunity: Th1 and Th2. The Th1 cells, a functional subset of CD4+ cells, are characterized by their ability to boost cell mediated immunity. The Th1 cell produces cytokines IL-2 and interferon- $\gamma$ , and are identified by the absence of IL-10, IL-4, IL-5 and IL-6.

The Th2 cell is also a CD4+ cell, but is distinct from the Th1 cell. The Th2 cells are responsible for antibody production and produce the cytokines IL-4, IL-5, IL-10 and IL-13. (see Figure 1). These cytokines play an important role in making the Th1 and Th2 responses mutually inhibitory. The interferon- $\gamma$  that is produced by the Th1 cells inhibits the proliferation of Th2 cells (Figure 2) while IL-10 produced by the Th2 cells represses the production of interferon- $\gamma$  (Figure 2).

Members of the four helical bundle cytokine family (Bazan, J. F., 1990, *Proc Natl Acad Sci U S A*, 87:6934-8) have been found to play a critical role in the expansion and terminal differentiation of T helper cells from a common precursor into distinct populations of Th1 and Th2 effector cells. O'Garra, A., 1998, *Immunity*, 8:275-83. IL-4 influence predominantly the development of Th2 cells while IL-12 is a major factor involved in the differentiation of Th1 cells. Hsieh, C. S., *et al.*, 1993, *Science*, 260:547-9; Seder, R. A., *et al.*, 1993, *Proc Natl Acad Sci U S A*, 90:10188-92; Le Gros, G., *et al.*, 1990, *J Exp Med*, 172:921-9; Swain, S. L., *et al.*, 1991, *Immunol Rev*, 123:115-44. Accordingly, mice deficient in IL-4 (Kuhn, R., *et al.*, 1991, *Science*, 254:707-10), IL-4 receptor chain (Noben-Trauth, N., *et al.*, 1997, *Proc Natl Acad Sci U S A*, 94:10838-43), or the IL-4 specific transcription factor STAT6 (Shimoda, K., *et al.*, 1996, *Nature*, 380:630-3) are defective in Th2 responses, while mice deficient in IL-12 (Magrath, J., *et al.*, 1996, *Immunity*, 4:471-81), IL-12 receptor (IL-12R)  $\beta$  chain (Wu, C., *et al.*, 1997, *J Immunol*, 159:1658-65), or the IL-12 specific transcription factor STAT4 (Kaplan, M. H., *et al.*, 1996, *Nature*, 382:174-7) have impaired Th1 responses.

Th-1 and Th-2 cell subtypes are believed to be derived from the common precursor, termed a Th-0 cell. In contrast to the mutually exclusive cytokine production of the Th-1 and Th-2 subtypes, Th-0 cells produce most or all of these cytokines. The release profiles of the different cytokines for the Th-1 and Th-2 subtypes plays an active role in the selection of effector mechanisms and cytotoxic cells. The IL-2 and  $\gamma$ -interferon secreted by Th-1 cells tends to activate macrophages and cytotoxic cells, while the IL-4, IL-5, IL-6 and IL-10 secreted by Th-2 cells tends to increase the production of eosinophils and mast cells as well as enhance the production of antibodies including IgE and decrease the function of cytotoxic cells. Once established, the Th-1 or Th-2 response pattern is maintained by the production of cytokines that inhibit the production of the other subset. The  $\gamma$ -interferon produced by Th-1 cells inhibits production of Th-2 cytokines such as IL-4 and IL-10, while the IL-10 produced by Th-2 cells inhibits the production of Th-1 cytokines such as IL-2 and  $\gamma$ -interferon.

The upset of the delicate balance between the cytokines produced by the Th1 and Th2 cell subsets leads to a host of disorders. For example, the overproduction of Th1 cytokines can lead to autoimmune inflammatory diseases, multiple sclerosis and inflammatory bowel disease (*e.g.*, Crohn's disease, regional enteritis, distal ileitis, granulomatous enteritis, regional ileitis, terminal ileitis). Similarly, overproduction of Th2 cytokines leads to allergic disorders, including anaphylactic hypersensitivity, asthma, allergic rhinitis, atopic dermatitis, vernal conjunctivitis, eczema, urticaria and food allergies. Umetsu *et al.*, *Soc. Exp. Biol. Med.* 215: 11-20 (1997).

WO 97/44455 filed 19 May 1997 and Sprecher *et al.*, *Biochem. Biophys. Res. Commun.* 246: 82-90 (1998) describe cytokine receptor molecules possessing a certain degree of sequence identity with the murine and

human TCCR molecules herein. The murine and human prior art cytokine receptors are purported to be expressed in lymphoid tissue, including the thymus, spleen, lymph nodes and peripheral blood leukocytes - and are further indicated to be present on both B- and T-cells and have a function relating to the proliferation, differentiation and/or activation of immune cells, perhaps in the development and regulation of the immune response. However, WO97/44455 and Sprecher *et al.*, *supra* identify neither the precise role of TCCR and its homologs in the mediation of T-cell differentiation and cytokine release profiles into Th1 subtype and Th2 subtype, nor the host of disorders that are implicated by the release of the cytokine T-cell subtypes.

#### Summary of the Invention

The present invention concerns methods for the diagnosis and treatment of immune related disease in mammals, including humans - specifically the physiology (*e.g.*, cytokine release profiles) and diseases resulting from a bias in the T-cell differentiation pathway into the Th1 subtype or the Th2 subtype. The present invention is based on the identification of the gene encoding and amino acid sequence of TCCR (previously known as NPOR), the absence or inactivation of which biases the differentiation of T-cells into the Th2 subtype in mammals. Certain immune diseases can be treated by suppressing or enhancing the differentiation of T-cells into either the Th1 or the Th2 subtype.

The present invention further concerns a method for enhancing, stimulating or potentiating the differentiation of T-cells into the Th2 subtype instead of the Th1 subtype, comprising the administration of an effective amount of a TCCR antagonist. Optionally, the method occurs in a mammal and the effective amount is a therapeutically effective amount. Optionally, the TCCR antagonist induced differentiation of T-cells into Th2 subtype cells further results in a Th2 cytokine release profile upon antigen stimulation (*e.g.*, IL-4, IL-5 IL-10 and IL-13). Diseases which are characterized by an overproduction of Th1 cytokines, and which would be responsive to the equilibrating effect of Th2-subtype stimulation of differentiation and the resulting cytokine release profile, include autoimmune inflammatory diseases (*e.g.*, allergic encephalomyelitis, multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune uveoretinitis, inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis), autoimmune thyroid disease) and allograft rejection.

The present invention further concerns a method for preventing, inhibiting or attenuating the differentiation of T-cells into the Th2 subtype (*i.e.*, causes differentiation into Th1 subtypes), comprising the administration of an effective amount of a TCCR or agonist. Optionally, the method occurs in a mammal and the effective amount is a therapeutically effective amount. Optionally, this TCCR or agonist induced differentiation results in a Th1 cytokine release profile upon antigen stimulation (*e.g.*,  $\gamma$ -interferon). Diseases which are characterized by an overproduction of Th2 cytokines (or insufficient production of Th1 cytokines), and which would be responsive to the equilibrating effect of Th1-subtype stimulation of differentiation Th2 cytokine overproduction would be expected to be effective in treating infectious diseases (*e.g.*, *Leishmania major*, *Mycobacterium leprae*, *Candida albicans*, *Toxoplasma gondi*, respiratory syncytial virus, human immunodeficiency virus) and allergic disorders (*e.g.*, asthma, allergic rhinitis, atopic dermatitis, vernal conjunctivitis).

In one embodiment, the present invention concerns an isolated antibody which binds a TCCR polypeptide (*e.g.*, anti-TCCR). In one aspect, the antibody mimics the activity of a TCCR polypeptide (an agonist antibody) or conversely the antibody inhibits or neutralizes the activity of a TCCR polypeptide (an antagonist antibody). In



another aspect, the antibody is a monoclonal antibody, which preferably has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues. The antibody may be labeled and may be immobilized on a solid support. In a further aspect, the antibody is an antibody fragment, a single-chain antibody, or an anti-idiotypic antibody.

5 In another embodiment, the invention concerns the use of the polypeptides and antibodies of the invention to prepare a composition or medicament which has the uses described above.

In a further embodiment, the invention concerns nucleic acid encoding an anti-TCCR antibody, and vectors and recombinant host cells comprising such nucleic acid. In a still further embodiment, the invention concerns a method for producing such an antibody by culturing a host cell transformed with nucleic acid encoding the antibody under conditions such that the antibody is expressed, and recovering the antibody from the cell culture.

10 The invention further concerns antagonists of a TCCR polypeptide that inhibit one or more functions or activities of the TCCR polypeptide. Alternatively, the invention concerns TCCR agonists that stimulate or enhance one or more functions or activities of the TCCR polypeptide. Preferably such antagonists and/or agonists are TCCR variants, peptide fragments, small molecules, antisense oligonucleotides (DNA or RNA), ribozymes or antibodies (monoclonal, humanized, specific, single-chain, heteroconjugate or fragment of the aforementioned). Additionally, TCCR agonists can include potential TCCR ligands, while potential TCCR antagonists can include soluble TCCR extracellular domains (ECD).

In a further embodiment, the invention concerns isolated nucleic acid molecules that hybridize to the nucleic acid molecules encoding the TCCR polypeptides, or the complement. The nucleic acid preferably is DNA, and hybridization preferably occurs under stringent conditions. Such nucleic acid molecules can act as antisense molecules of the amplified genes identified herein, which, in turn, can find use in the modulation of the respective amplified genes, or as antisense primers in amplification reactions. Furthermore, such sequences can be used as part of ribozyme and/or triple helix sequence which, in turn, may be used in regulation of the amplified genes.

20 In another embodiment, the invention concerns a method for determining the presence of a TCCR polypeptide comprising exposing a cell suspected of containing the polypeptide to an anti-TCCR antibody and determining the binding of the antibody to the cell.

In yet another embodiment, the present invention concerns a method of diagnosing a Th1-mediated or Th2-mediated disorder in a mammal, comprising detecting the level of expression of a gene encoding a TCCR polypeptide (a) in a test sample of tissue cells obtained from the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a lower expression level in the test sample versus the control indicates the presence of a Th2-mediated disorder and a higher expression level in the test sample versus the control indicates the presence of a Th1-mediated disorder in the mammal from which the test tissue cells were obtained.

30 In another embodiment, the present invention concerns a method of diagnosing an immune disease in a mammal, comprising (a) contacting an anti-TCCR antibody with a test sample of tissue cells obtained from the mammal, and (b) detecting the formation of a complex between the antibody and the TCCR polypeptide in the test sample. The detection may be qualitative or quantitative, and may be performed in comparison with monitoring the complex formation in a control sample of known normal tissue cells of the same cell type. A larger quantity of complexes formed in the test sample indicates the presence of TCCR and a Th1-mediated disorder, while a lesser

quantity indicates a Th2-mediated disorder in the mammal from which the test tissue cells were obtained. The antibody preferably carries a detectable label. Complex formation can be monitored, for example, by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. The test sample is usually obtained from an individual suspected of having a deficiency or abnormality of the immune system.

5 In another embodiment, the present invention concerns a diagnostic kit, containing an anti-TCCR antibody and a carrier (*e.g.* a buffer) in suitable packaging. The kit preferably contains instructions for using the antibody to detect the TCCR polypeptide.

In a further embodiment, the invention concerns an article of manufacture, comprising:

a container;

10 a label on the container; and

a composition comprising an active agent contained within the container; wherein the composition is effective for stimulating or inhibiting an immune response in a mammal, the label on the container indicates that the composition can be used to treat an immune related disease, and the active agent in the composition is an agent stimulating or inhibiting the expression and/or activity of the TCCR polypeptide. In a preferred aspect, the active agent is a TCCR polypeptide or an anti-TCCR antibody.

15 A further embodiment is a method for identifying a compound capable of modulating the expression and/or biological activity of a TCCR polypeptide by contacting a candidate compound with a TCCR polypeptide under conditions and for a time sufficient to allow these two components to interact. In a specific aspect, either the candidate compound or the TCCR polypeptide is immobilized on a solid support. In another aspect, the non-immobilized component carries a detectable label.

#### **Brief Description of the Drawings**

Figure 1 is a diagrammatic representation of the differentiation of the CD4+ T-cell differentiation into Th1 and Th2 cells, the primary cytokines responsible for effecting the differentiation, the primary cytokines released from the differentiation of the respective subsets upon antigen stimulation and the physiological effects mediated by the cytokine profiles released.

25 Figure 2 is a diagrammatic representation of the negative feedback loop describing the interrelationship between the cytokines released by the Th1 and Th2 T-cell subtypes.

Figure 3 shows the amino acid sequence for human TCCR (hTCCR) (SEQ ID NO:1). The sequence has also been published in WO97/44455 filed on 23 May 1996 and is further available from GenBank under accession number 4759327. This sequence is further described in Sprecher *et al.*, *Biochem. Biophys. Res. Commun.* **246**(1): 82-90 (1998). In SEQ ID NO:1, a signal peptide has been identified from amino acid residues 1 to about 32, a transmembrane domain from about amino acid residues 517 to about 538, N-glycosylation sites at about residues 51-54, 76-79, 302-305, 311-314, 374-377, 382-385, 467-470, 563-566, N-myristoylation sites at about residues 107-112, 240-245, 244-249, 281-286, 292-297, 373-378, 400-405, 459-464, 470-475, 531-536 and 533-538, a prokaryotic membrane lipoprotein lipid attachment site at about residues 522-532 and a growth factor and cytokine receptor family signature 1 at about residues 41-54. There is also a region of significant homology with the second subunit of the receptor for human granulocyte-macrophage colony-stimulating factor (GM-CSF) at residues 183-191.

Figure 4 shows the amino acid sequence for murine TCCR (mTCCR) (SEQ ID NO:2). The sequence has also been published in WO97/44455 filed on 23 May 1996 and is further available from GenBank under accession number 7710109. This sequence is further described in Sprecher *et al.*, *Biochem. Biophys. Res. Commun.* 246(1): 82-90 (1998). In SEQ ID NO:2, a signal peptide has been identified from amino acid residues 1 to about 24, the transmembrane domain from about amino acid residues 514 to about 532, N-glycosylation sites at about residues, 46-49, 296-299, 305-308, 360-361, 368-371 and 461-464, casein kinase II phosphorylation sites at about residues 10-13, 93-96, 130-133, 172-175, 184-187, 235-238, 271-274, 272-275, 323-326, 606-609 and 615-618, a tyrosine kinase phosphorylation site at about residues 202-209, N-myristoylation sites at about residues 43-48, 102-107, 295-300, 321-326, 330-335, 367-342, 393-398, 525-530 and 527-532, an amidation site at about residues 240-243, a prokaryotic membrane lipoprotein lipid attachment at about residues 516-526 and a growth factor and cytokine receptor family signature 1 at about residues 36-49. Region of significant homology exist with: (1) human erythropoietin at about residues 14-51 and (2) murine interleukin-5 receptor at residues 211-219.

Figure 5 is a comparison of hTCCR (SEQ ID NO:1) and mTCCR (SEQ ID NO:2). Identical amino acids are shaded and gaps introduced for optimal alignment are indicated by dashes. The predicted signal peptidase cleavage site is indicated by an arrowhead. Potential N-glycosylation sites are indicated with an asterisk. The WSX motif, transmembrane domain and box1 motif are boxed.

Figure 6 is a Northern blot of human TCCR indicating the expression profiles in adult and fetal tissues. In adults, hTCCR is most highly expressed in the thymus, but there is also signal in peripheral blood leukocytes (PBL's), spleen as well as weak expression in the lung. In fetal tissues, TCCR exhibits weak expression in lung and kidney. The expression profile of TCCR indicates that it may be involved in blood cell development and proliferation, especially of thymocytes.

Figure 7(A-B) examines the number and phenotype of T-cells in TCCR <sup>-/-</sup> mice. Figure 7A is a contour plot of FACS analysis of CD4<sup>+</sup>/CD8<sup>+</sup> T-cells taken from TCCR <sup>-/-</sup> mice and compared with wild type. Figure 7B is a contour plot of FACS analysis of CD4<sup>+</sup>/CD8<sup>+</sup>/TcR<sup>+</sup>. The lack of any significant difference between the numbers of T-cells in TCCR <sup>-/-</sup> mice indicates that T-cell proliferation is not impaired.

Figure 8(A-B) examines the expression of TCCR on human T-cells. Figure 8A is a FACS analysis contour plot of human TCCR and the pan T-cell surface marker CD2 on human T-cells. Figure 8B is a FACS analysis contour plot of human TCCR and the B-cell marker CD20 on human B-cells. The left-most plot of both figures represent the appropriate flouochrome conjugated secondary antibody. Cumulatively, Figures 8A and 8B indicate that TCCR is found on a subset of human T-cells and is not present in appreciable amounts on B-cells.

Figure 9(A-C) is a diagrammatic representation of the TCCR gene targeting methodology using homologous recombination. Figure 9A represents the wild type allele with the TCCR exons denoted by solid blocks and the introns as intervening lines. "E" and "B" indicate cleavage sites for the endonucleases EcoRI and BamHI, respectively. Figure 9B represents the targeting vector wherein exons 3-8 of TCCR have been replaced with the neomycin resistance gene from the plasmid vector pGK-neo. The thymidine kinase gene from herpes simplex virus has been inserted 5' to exon 1, a gene which provides resistance to selective pressure from gancyclovir. Figure 9C is a representation of the final targeted or "knockout" allele after homologous recombination between the endogenous gene and the targeting vector has occurred.

Figures 10(A-C) are a Southern blot, gel electrophoresis image of PCR reaction and a Northern blot, respectively confirming transfection with the TCCR targeting vector. In Figure 10A, genomic DNA was taken from ES cells resistant to the Neomycin/Gancyclovir drug selection and hybridized with a radiolabeled probe specific for TCCR. In the second lane from the left, the existence of both a 10 Kb and a 12 Kb fragment indicates that one of the TCCR alleles has been ablated. Figure 10B is the reaction product of PCR amplified genomic DNA from TCCR  $-/-$  mouse tails. The PCR primers were designed so as differentiate between the wild type TCCR allele and the targeted ("knockout") allele resulting from the recombination event. Lanes 1 and 2 (counted from the left) show a band pattern indicative of TCCR wild type. Lane 3 shows a PCR band from a TCCR  $-/-$  mouse and lanes 5 and 6 are indicative of a TCCR heterozygote mouse (+/-). Figure 10C is a Northern blot that has been hybridized with a probe specific for TCCR. Lane 1 is from a TCCR  $-/-$  mouse and lane 2 is from a wild type mouse. The lack of any signal from the TCCR  $-/-$  mouse indicates that there is no functional full length mRNA of TCCR being produced

Figure 11(A-B) indicates an enhancement of allergic airway inflammation in TCCR  $-/-$  mice. Figure 11A shows that TCCR  $-/-$  mice sensitized with Dust Mite Antigen (DMA) produce a greater Th2 response as measured by the number of lymphocytes that infiltrate the lung.

Figure 12(A-B) is a graphical representation of the Th1/Th2 responses in TCCR  $-/-$  mice, as measured by production of IFN- $\gamma$ . In Figure 12A, T-cells isolated from TCCR  $-/-$  mice are incubated with IL-12 which causes differentiation along the Th1 pathway. These cells were assayed for their production of IFN- $\gamma$ , IL-4 and IL-5. IFN- $\gamma$  is produced at significantly lower levels in the TCCR  $-/-$  mice as indicated by the lighter shaded bars in Figure 12A. This indicates a greatly weakened Th1 response in the TCCR  $-/-$  mice. Figure 12B is a graphical representation of T-cells that have been incubated with IL-4 which causes differentiation along the Th2 pathway. This indicates no difference in cytokine production between the TCCR  $-/-$  mice T-cells and wild type control cells.

Figure 13 is a graphical representation of Ig levels produced in TCCR  $-/-$  mice. Levels of Ig subtypes IgG1, IgG2, IgG2b, IgG3, IgM and IgA were examined. As indicated by the lighter shadowed bars, TCCR  $-/-$  mice produced less IgG2a than wild type controls. The rest of the IgG levels did not differ significantly. IgG2a is produced by Th1 cells, and its notable absence in the TCCR  $-/-$  mice confirms the reduced Th1 response observed in other assays presented herein.

Figure 14 is a graphical representation of IgG levels produced in TCCR  $-/-$  mice that have been previously immunized with ovalbumin. Mice were injected with 100 $\mu$ g OVA ip on day 1 and 21 then bled on day 26. Levels of IgG1 and IgG2a were measured in the homozygous knockout mice compared to the wild type. As shown in the left side of the graph, IgG1 levels were equivalent in the wild type and knockout, whereas IgG2a levels were significantly lower in the TCCR  $-/-$  knockout compared to the wild type, reflecting a weakened Th1 response in TCCR  $-/-$  mice.

Figure 15(A-B) is a graphical representation showing which cell types within murine splenocytes express TCCR. Figure 15A shows expression levels in CD4, CD8, CD19, NK1.1 and F4/80 cells, with highest levels in CD4 T cells and natural killer cells. Figure 15B shows expression levels within Th0, Th1 and Th2 cells, with expression being highest in Th0 cells and down-regulated upon differentiation of CD4 cells in both Th1 and Th2 cells. TCCR expression was detected by real time PCR and normalized to rpl19, a ribosomal housekeeping gene. Heid, C.A., *et al.*, 1996, *Genome Res.*, 6:986-94.

Figure 16(A-D) is a graphical representation of antigen induced cytokine production and proliferation by lymph node cells from TCCR-deficient mice. Wild type and TCCR-deficient mice were immunized with KLH in complete Freund's adjuvant (CFA). Lymph nodes were harvested 9 days later and cultured in the presence of KLH as indicated and analyzed for their capacity to produce (Figure 16A) IFN $\gamma$ , (Figure 16B) IL-4, (Figure 16C) IL-5 or (Figure 16D) to proliferate. Data are presented as the mean  $\pm$  SD values that were derived from 5 animals in each group.  $P < 0.004$  by unpaired T-test for IFN $\gamma$  levels between WT and KO at both KLH concentrations.

Figure 17(A-C) is a graphical representation of the effect on IgG subclass concentrations and sensitivity to *L. monocytogenes* infection. Serum was collected from wild type and TCCR-deficient mice, and total IgG subclass concentrations was determined by ELISA (Figure 17A). OVA-specific IgG1 and IgG2a from OVA/CFA primed mice. Serum was collected from wild type and TCCR-deficient mice that were immunized with OVA in CFA and levels of IgG1 (1:320000 dilution) and IgG2a (1:5000 dilution) were determined by OVA-specific ELISA (Figure 17B). Five TCCR-deficient mice or wild type littermates were infected subcutaneously with  $3 \times 10^4$  CFU of *L. monocytogenes*. Three or nine days later, the livers were harvested and bacterial titers were determined (Figure 17C). Data are presented as the mean  $\pm$  SD values that were derived from 5 animals in each group.  $P < 0.001$  by unpaired T-test between WT and KO at both time points.

Figure 18(A-D) is a graphical representation of the *in vitro* induction of Th cell differentiation and proliferation. CD4 $^{+}$  T-cells purified from the spleens of wild type or TCCR-deficient mice were differentiated into Th1 or Th2 cells (Figure 18A) in the presence of ConA and irradiated wild type APC or (Figure 18B) with anti-CD3 and anti-CD28 as stimuli. Production of IFN $\gamma$  and IL-4 was determined by ELISA. Data represent the mean value  $\pm$  SD of pools of 5 mice per group. ND, not detected. Figure 18C represents IL-12 induced proliferation of splenocytes from wild type and TCCR-deficient mice. ConA activated splenocytes were incubated for 24h in the presence of increasing concentrations of IL-12 as indicated. Proliferation of cells was measured by incorporation of [3H]-thymidine during the final 6h. Figure 18D represents IL-12R mRNA levels in unstimulated (white bars) and ConA stimulated (black bars) splenocytes. Splenic T-cells were stimulated with ConA for 72h and mRNA levels for IL-12R  $\alpha$  and IL-12R  $\beta$  were determined by real time quantitative PCR (Taqman). Fold increase are relative to the levels of RNA present in wild type unstimulated cells.

Figure 19 shows the sequences of SEQ ID NOS:5-16 which represent the primers and probes that were used with the Taqman analysis.

### **Detailed Description of the Preferred Embodiments**

#### **I. Definitions**

The term "immune related disease" means a disease in which a component of the immune system of a mammal causes, mediates or otherwise contributes to a morbidity in the mammal. Also included are diseases in which stimulation or intervention of the immune response has an ameliorative effect on progression of the disease. Included within this term are immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases, infectious diseases, immunodeficiency diseases, neoplasia, *etc.*

The term "Th1 mediated disorder" means a disease which is characterized by the overproduction of Th1 cytokines, including those that result from an overproduction or bias in the differentiation of T-cells into the Th1 subtype. Such diseases include, for example, autoimmune inflammatory diseases (*e.g.*, allergic encephalomyelitis,

multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune uveoretinitis, thyrotoxicosis, scleroderma, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis, regional enteritis, distal ileitis, granulomatous enteritis, regional ileitis, terminal ileitis), autoimmune thyroid disease, pernicious anemia) and allograft rejection.

5           The term "Th2 mediated disorder" means a disease which is characterized by the overproduction of Th2 cytokines, including those that result from an overproduction or bias in the differentiation of T-cells into the Th2 subtype. Such diseases include, for example, exacerbation of infection with infectious diseases (*e.g.*, *Leishmania major*, *Mycobacterium leprae*, *Candida albicans*, *Toxoplasma gondi*, respiratory syncytial virus, human immunodeficiency virus, *etc.*) and allergic disorders, such as anaphylactic hypersensitivity, asthma, allergic rhinitis, atopic dermatitis, vernal conjunctivitis, eczema, urticaria and food allergies, *etc.*

10           Examples of other immune, immune-related and inflammatory diseases, some of which are mediated by the effects (*e.g.*, cytokine release profiles) of differentiation of T-cells into the Th1 and Th2 subtypes, and which can be treated according to the invention include, systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis (scleroderma), idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis) autoimmune inflammatory diseases (*e.g.*, allergic encephalomyelitis, multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune uveoretinitis, thyrotoxicosis, scleroderma, systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease (*e.g.*, Crohn's disease, ulcerative colitis, regional enteritis, distal ileitis, granulomatous enteritis, regional ileitis, terminal ileitis), autoimmune thyroid disease, pernicious anemia) and allograft rejection, diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease (ulcerative colitis, Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft-versus-host-disease. Infectious diseases including viral diseases such as AIDS (HIV infection), hepatitis A, B, C, D, and E, herpes, *etc.*, bacterial infections, fungal infections, protozoal infections, parasitic infections, and respiratory syncytial virus, human immunodeficiency virus, *etc.*) and allergic disorders, such as anaphylactic hypersensitivity, asthma, allergic rhinitis, atopic dermatitis, vernal conjunctivitis, eczema, urticaria and food allergies, *etc.*

35           "Treatment" is an intervention performed with the intention of preventing the development or altering the pathology of a disorder. Accordingly, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent, slow down (lessen) or ameliorate the targeted pathological condition or

disorder. Those in need of treatment include those already with the disorder as well as those in which the disorder is to be prevented. In treatment of an immune related disease (*e.g.*, Th1-mediated and Th2-mediated disorder), a therapeutic agent may directly decrease or increase the magnitude of response of a pathological component of the disorder, or render the disease more susceptible to treatment by other therapeutic agents, *e.g.* antibiotics, antifungals, anti-inflammatory agents, chemotherapeutics, *etc.*

The term "effective amount" is the minimum concentration of TCCR polypeptide, agonist thereof and/or antagonist thereof which causes, induces or results in either a detectable bias in the differentiation of T-cells into either the Th1 subtype or the Th2 subtype and/or the cytokine release profile which these T-cell subtypes secrete. Furthermore a "therapeutically effective amount" is the minimum concentration (amount) of TCCR polypeptides, agonists thereof and/or antagonist thereof which would be effective in treating either Th1-mediated or Th2-mediated disorders.

"Chronic" administration refers to administration of the agent(s) in a continuous mode as opposed to an acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "Intermittent" administration is treatment that is not consecutively done without interruption, but rather is cyclic in nature.

The "pathology" of an immune related disease includes all phenomena that compromise the well-being of the patient. This includes, without limitation, abnormal or uncontrollable cell growth, antibody production, auto-antibody production, complement production and activation, interference with the normal functioning of neighboring cells, release of cytokines or other secretory products at abnormal levels, suppression or aggravation of any inflammatory or immunological response, infiltration of inflammatory cells (neutrophilic, eosinophilic, monocytic, lymphocytic) into tissue spaces, *etc.*

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cattle, sheep, pigs, goats, rabbit, *etc.* Preferably, the mammal is human.

Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

"Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN<sup>TM</sup>, polyethylene glycol (PEG), and PLURONICS<sup>TM</sup>.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.* I<sup>131</sup>, I<sup>125</sup>, Y<sup>90</sup> and Re<sup>186</sup>), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, especially cancer cell overexpressing any of the genes identified herein, either *in vitro* or *in vivo*. Thus, the growth inhibitory agent is one which significantly reduces the percentage of cells overexpressing such genes in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogens, and antineoplastic drugs" by Murakami *et al.* (WB Saunders: Philadelphia, 1995), especially p. 13.

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- $\alpha$  and - $\beta$ ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- $\beta$ ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- $\alpha$  and TGF- $\beta$ ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- $\alpha$ , - $\beta$ , and - $\gamma$ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 $\alpha$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; a tumor necrosis factor such as TNF- $\alpha$  or TNF- $\beta$ ; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

The terms "TCCR polypeptide", "TCCR protein" and "TCCR" when used herein encompass native sequence TCCR and TCCR polypeptide variants (which are further defined herein). The TCCR polypeptide may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods.

A "native sequence TCCR" comprises a polypeptide having the same amino acid sequence as a TCCR polypeptide derived from nature. Such native sequence TCCR can be isolated from nature or can be produced by recombinant and/or synthetic means. The term "native sequence TCCR" specifically encompasses naturally-occurring truncated or secreted forms (*e.g.*, an extracellular domain sequence), naturally-occurring truncated forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants of the TCCR. In one embodiment of the invention, the native sequence human TCCR is a mature or full-length native sequence TCCR comprising amino acids 1 to 636 of Figure 3 (SEQ ID NO:1). Similarly, the native sequence murine TCCR is a mature or full-length native sequence TCCR comprising amino acid 1 to 623 of Figure 4 (SEQ ID NO:2). Also, while the TCCR polypeptides disclosed in



Figure 3 (SEQ ID NO:1) and Figure 4 (SEQ ID NO:2) is shown to begin with the methionine residue designated herein as amino acid position 1, it is conceivable and possible that another methionine residue located either upstream or downstream from amino acid position 1 in Figure 3 (SEQ ID NO:1) or Figure 4 (SEQ ID NO:2) may be employed as the starting amino acid residue for the TCCR polypeptide.

5           The "TCCR polypeptide extracellular domain" or "TCCR ECD" refers to a form of the TCCR polypeptide which is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a TCCR polypeptide ECD will have less than about 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than about 0.5% of such domains. It will be understood that any transmembrane domain(s) identified for the TCCR polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely be no more than about 5 amino acids at either end of the domain as initially identified. As such, in one embodiment of the present invention, the extracellular domain of a human TCCR polypeptide comprises amino acids 1 or about 33 to X<sub>1</sub> wherein X<sub>1</sub> is any amino acid residue from residue 512 to residue 522 of Figure 3 (SEQ ID NO:1). Similarly, the extracellular domain of the murine TCCR polypeptide comprises amino acids 1 or about 25 to X<sub>2</sub> wherein X<sub>2</sub> is any amino acid residues from residue 509 to residue 519 of Figure 4 (SEQ ID NO:2).

15           "TCCR variant polypeptide" means an active TCCR polypeptide as defined below having at least about 80% amino acid sequence identity with the amino acid sequence of: (a<sub>1</sub>) residue 1 or about 33 to 636 of the human TCCR polypeptide shown in Figure 3 (SEQ ID NO:1); (a<sub>2</sub>) residue 1 or about 25 to 623 of the murine TCCR polypeptide shown in Figure 4 (SEQ ID NO:2); (b<sub>1</sub>) X<sub>3</sub> to 636 of the human TCCR polypeptide shown in Figure 3 (SEQ ID NO:1), wherein X<sub>3</sub> is any amino acid residue 27 to 37 of Figure 3 (SEQ ID NO:1); (b<sub>2</sub>) X<sub>4</sub> to 623 of the murine TCCR polypeptide shown in Figure 4 (SEQ ID NO:2), wherein X<sub>4</sub> is any amino acid residue from 20 to 30 of Figure 4 (SEQ ID NO:2); (c<sub>1</sub>) 1 or about 33 to X<sub>1</sub>, wherein X<sub>1</sub> is any amino acid residue from residue 512 to residue 522 and of Figure 3 (SEQ ID NO:1); (c<sub>2</sub>) 1 or about 25 to X<sub>2</sub>, wherein X<sub>2</sub> is any amino acid residue from residue 509 to 519 of Figure 4 (SEQ ID NO:2); (d<sub>1</sub>) X<sub>5</sub> to 636, wherein X<sub>5</sub> is any amino acid from residue 533 to 543 of Figure 3 (SEQ ID NO:1); (d<sub>2</sub>) X<sub>6</sub> to 623, wherein X<sub>6</sub> is any amino acid from residue 527 to 537 of Figure 4 (SEQ ID NO:2) or (e) another specifically derived fragment of the amino acid sequences shown in Figure 3 (SEQ ID NO:1) and in Figure 4 (SEQ ID NO:2).

Such TCCR variant polypeptides include, for instance, TCCR polypeptides wherein one or more amino acid residues are added, or deleted, at the N- and/or C-terminus, as well as within one or more internal domains, of the sequence of Figure 3 (SEQ ID NO:1) and Figure 4 (SEQ ID NO:2). Ordinarily, a TCCR variant polypeptide will have at least about 80% amino acid sequence identity, more preferably at least about 81% amino acids sequence identity, more preferably at least about 82% amino acid sequence identity, more preferably at least about 83% amino acid sequence identity, more preferably at least about 84% amino acid sequence identity, more preferably at least about 85% amino acid sequence identity, more preferably at least about 86% amino acid sequence identity, more preferably at least about 87% amino acid sequence identity, more preferably at least about 88% amino acid sequence identity, more preferably at least about 89% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity, more preferably at least about 91% amino acid sequence identity, more preferably at least about 92% amino acid sequence identity, more preferably at least about 93% amino acid sequence identity, more preferably

at least about 94% amino acid sequence identity, more preferably at least about 95% amino acid sequence identity, more preferably at least about 96% amino acid sequence identity, more preferably at least about 97% amino acid sequence identity, more preferably at least about 98% amino acid sequence identity, more preferably at least about 99% amino acid sequence identity with: (a<sub>1</sub>) residue 1 or about 33 to 636 of the human TCCR polypeptide shown in Figure 3 (SEQ ID NO:1); (a<sub>2</sub>) residue 1 or about 25 to 623 of the murine TCCR polypeptide shown in Figure 4 (SEQ ID NO:2); (b<sub>1</sub>) X<sub>3</sub> to 636 of the human TCCR polypeptide shown in Figure 3 (SEQ ID NO:1), wherein X<sub>3</sub> is any amino acid residue 27 to 37 of Figure 3 (SEQ ID NO:1); (b<sub>2</sub>) X<sub>4</sub> to 623 of the murine TCCR polypeptide shown in Figure 4 (SEQ ID NO:2), wherein X<sub>4</sub> is any amino acid residue from 20 to 30 of Figure 4 (SEQ ID NO:2); (c<sub>1</sub>) 1 or about 33 to X<sub>1</sub> wherein X<sub>1</sub> is any amino acid residue from residue 512 to residue 522 and of Figure 3 (SEQ ID NO:1); (c<sub>2</sub>) 1 or about 25 to X<sub>2</sub>, wherein X<sub>2</sub> is any amino acid residue from residue 509 to 519 of Figure 4 (SEQ ID NO:2); (d<sub>1</sub>) X<sub>5</sub> to 636, wherein X<sub>5</sub> is any amino acid from residue 533 to 543 of Figure 3 (SEQ ID NO:1); (d<sub>2</sub>) X<sub>6</sub> to 623, wherein X<sub>6</sub> is any amino acid from residue 527 to 537 of Figure 4 (SEQ ID NO:2) or (e) another specifically derived fragment of the amino acid sequences shown in Figure 3 (SEQ ID NO:1) and in Figure 4 (SEQ ID NO:2).

TCCR variant polypeptides are at least about 10 amino acids in length, often at least about 20 amino acids in length, more often at least about 30 amino acids in length, more often at least about 40 amino acids in length, more often at least about 50 amino acids in length, more often at least about 60 amino acids in length, more often at least about 70 amino acids in length, more often at least about 80 amino acids in length, more often at least about 90 amino acids in length, more often at least about 100 amino acids in length, more often at least about 150 amino acids in length, more often at least about 200 amino acids in length, more often at least about 250 amino acids in length, more often at least about 300 amino acids in length, more often at least about 400 amino acids in length, more often at least about 500 amino acids in length, more often at least about 600 amino acids in length, or more.

"Percent (%) amino acid sequence identity" with respect to the polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in a sequence of the TCCR polypeptides, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are obtained as described below by using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 3(A-Q). The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 3(A-Q) has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 3(A-Q). The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

For purposes herein, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

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$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. As examples of % amino acid sequence identity calculations, Table 2(A-B) demonstrate how to calculate the % amino acid sequence identity of the amino acid sequence designated "Comparison Protein" to the amino acid sequence designated "PRO".

Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described above using the ALIGN-2 sequence comparison computer program. However, % amino acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institutes of Health, Bethesda, MD, USA 20892. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

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where X is the number of amino acid residues scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

Also included within the term "polypeptides of the invention" are polypeptides which in the context of the amino acid sequence identity comparisons performed as described above, include amino acid residues in the sequences compared that are not only identical, but also those that have similar properties. These polypeptides are termed "positives". Amino acid residues that score a positive value to an amino acid residue of interest are those that

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are either identical to the amino acid residue of interest or are a preferred substitution (as defined in Table I below) of the amino acid residue of interest. For purposes herein, the % value of positives of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % positives to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

where X is the number of amino acid residues scoring a positive value as defined above by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % positives of A to B will not equal the % positives of B to A.

"TCCR variant polynucleotide" or "TCCR variant nucleic acid sequence" means a nucleic acid molecule which encodes an active TCCR polypeptide as defined below and which has at least about 80% nucleic acid sequence identity with a nucleic acid sequence which encodes: (a<sub>1</sub>) amino acid residues 1 or about 33 to 636 of the human TCCR polypeptide shown in Figure 3 (SEQ ID NO:1); (a<sub>2</sub>) amino acid residues 1 or about 25 to 623 of the murine TCCR polypeptide shown in Figure 4 (SEQ ID NO:2); (b<sub>1</sub>) amino acids X<sub>3</sub> to 636 of the TCCR polypeptide shown in Figure 3 (SEQ ID NO:1), wherein X<sub>3</sub> is any amino acid residue from 27 to 37 of Figure 3 (SEQ ID NO:1); (b<sub>2</sub>) amino acids X<sub>4</sub> to 623 of the TCCR polypeptide shown in Figure 4 (SEQ ID NO:2), wherein X<sub>4</sub> is any amino acid residue from 20 to 30 of Figure 4 (SEQ ID NO:2); (c<sub>1</sub>) amino acids 1 or about 33 to X<sub>1</sub> wherein X<sub>1</sub> is any amino acid residue from residue 512 to residue 522 and of Figure 3 (SEQ ID NO:1); (c<sub>2</sub>) amino acids 1 or about 25 to X<sub>2</sub>, wherein X<sub>2</sub> is any amino acid residue from residue 509 to 519 of Figure 4 (SEQ ID NO:2); (d<sub>1</sub>) amino acids X<sub>5</sub> to 636, wherein X<sub>5</sub> is any amino acid from residue 533 to 543 of Figure 3 (SEQ ID NO:1); (d<sub>2</sub>) amino acids X<sub>6</sub> to 623, wherein X<sub>6</sub> is any amino acid from residue 527 to 537 of Figure 4 (SEQ ID NO:2); or (e) a nucleic acid sequence which encodes another specifically derived fragment of the amino acid sequence shown in Figure 3 (SEQ ID NO:1) or Figure 4 (SEQ ID NO:2). Ordinarily, a TCCR variant polynucleotide will have at least about 80% nucleic acid sequence identity, more preferably at least about 81% nucleic acid sequence identity, more preferably at least about 82% nucleic acid sequence identity, more preferably at least about 83% nucleic acid sequence identity, more preferably at least about 84% nucleic acid sequence identity, more preferably at least about 85% nucleic acid sequence identity, more preferably at least about 86% nucleic acid sequence identity, more preferably at least about 87% nucleic acid sequence identity, more preferably at least about 88% nucleic acid sequence identity, more preferably at least about 89% nucleic acid sequence identity, more preferably at least about 90% nucleic acid sequence identity, more preferably at least about 91% nucleic acid sequence identity, more preferably at least about 92% nucleic acid sequence identity, more preferably at least about 93% nucleic acid sequence identity, more preferably at least about 94% nucleic acid sequence identity, more preferably at least about 95% nucleic acid sequence identity, more preferably at least about 96% nucleic acid sequence identity, more preferably at least about 97% nucleic acid sequence identity, more preferably at least about 98% nucleic acid sequence identity and yet more preferably at least about 99% nucleic acid sequence identity with a nucleic acid sequence encoding amino acid residues: (a<sub>1</sub>) 1 or about 33 to 636 of the human

TCCR polypeptide shown in Figure 3 (SEQ ID NO:1); (a<sub>2</sub>) 1 or about 25 to 623 of the murine TCCR polypeptide shown in Figure 4 (SEQ ID NO:2); (b<sub>1</sub>) X<sub>3</sub> to 636 of the human TCCR polypeptide shown in Figure 3 (SEQ ID NO:1), wherein X<sub>3</sub> is any amino acid residue 27 to 37 of Figure 3 (SEQ ID NO:1); (b<sub>2</sub>) X<sub>4</sub> to 623 of the murine TCCR polypeptide shown in Figure 4 (SEQ ID NO:2), wherein X<sub>4</sub> is any amino acid residue from 20 to 30 of Figure 4 (SEQ ID NO:2); (c<sub>1</sub>) 1 or about 33 to X<sub>1</sub>, wherein X<sub>1</sub> is any amino acid residue from residue 512 to residue 522 and of Figure 3 (SEQ ID NO:1); (c<sub>2</sub>) 1 or about 25 to X<sub>2</sub>, wherein X<sub>2</sub> is any amino acid residue from residue 509 to 519 of Figure 4 (SEQ ID NO:2); (d<sub>1</sub>) X<sub>5</sub> to 636, wherein X<sub>5</sub> is any amino acid from residue 533 to 543 of Figure 3 (SEQ ID NO:1); (d<sub>2</sub>) X<sub>6</sub> to 623, wherein X<sub>6</sub> is any amino acid from residue 527 to 537 of Figure 4 (SEQ ID NO:2) or (e) another specifically derived fragment of the amino acid sequences shown in Figure 3 (SEQ ID NO:1) and in Figure 4 (SEQ ID NO:2).

Ordinarily, TCCR variant polynucleotides are at least about 30 nucleotides in length, often at least about 60 nucleotides in length, more often at least about 90 nucleotides in length, more often at least about 120 nucleotides in length, more often at least about 150 nucleotides in length, more often at least about 180 nucleotides in length, more often at least about 210 nucleotides in length, more often at least about 240 nucleotides in length, more often at least about 270 nucleotides in length, more often at least about 300 nucleotides in length, more often at least about 450 nucleotides in length, more often at least about 600 nucleotides in length, more often at least about 900 nucleotides in length, or more.

"Percent (%) nucleic acid sequence identity" with respect to the TCCR polypeptide-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in an invention polypeptide-encoding sequence of interest, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For purposes herein, however, % nucleic acid sequence identity values are obtained as described below by using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 3(A-Q). The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 3(A-Q) has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 3(A-Q). The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

For purposes herein, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

100 times the fraction  $W/Z$

where  $W$  is the number of nucleotides scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of  $C$  and  $D$ , and where  $Z$  is the total number of nucleotides in  $D$ . It will be appreciated that where the length of nucleic acid sequence  $C$  is not equal to the length of nucleic acid sequence  $D$ , the % nucleic acid sequence identity of  $C$  to  $D$  will not equal the % nucleic acid sequence identity of  $D$  to  $C$ . As examples of % nucleic acid sequence identity calculations, Table 2(C-D) demonstrates how to calculate the % nucleic acid sequence identity of the nucleic acid sequence designated "Comparison DNA" to the nucleic acid sequence designated "PRO-DNA".

Unless specifically stated otherwise, all % nucleic acid sequence identity values used herein are obtained as described above using the ALIGN-2 sequence comparison computer program. However, % nucleic acid sequence identity may also be determined using the sequence comparison program NCBI-BLAST2 (Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program may be downloaded from <http://www.ncbi.nlm.nih.gov> or otherwise obtained from the National Institutes of Health, Bethesda, MD USA 20892. NCBI-BLAST2 uses several search parameters, wherein all of those search parameters are set to default values including, for example, unmask = yes, strand = all, expected occurrences = 10, minimum low complexity length = 15/5, multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for final gapped alignment = 25 and scoring matrix = BLOSUM62.

In situations where NCBI-BLAST2 is employed for sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence  $C$  to, with, or against a given nucleic acid sequence  $D$  (which can alternatively be phrased as a given nucleic acid sequence  $C$  that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence  $D$ ) is calculated as follows:

100 times the fraction  $W/Z$

where  $W$  is the number of nucleotides scored as identical matches by the sequence alignment program NCBI-BLAST2 in that program's alignment of  $C$  and  $D$ , and where  $Z$  is the total number of nucleotides in  $D$ . It will be appreciated that where the length of nucleic acid sequence  $C$  is not equal to the length of nucleic acid sequence  $D$ , the % nucleic acid sequence identity of  $C$  to  $D$  will not equal the % nucleic acid sequence identity of  $D$  to  $C$ .

In other embodiments, TCCR variant polynucleotides are nucleic acid molecules that encode an active polypeptide of the invention and which are capable of hybridizing, preferably under stringent hybridization and wash conditions, to nucleotide sequences encoding the full-length invention polypeptide. Invention variant polypeptides include those that are encoded by an invention variant polynucleotide.

The term "positives", in the context of the amino acid sequence identity comparisons performed as described above, includes amino acid residues in the sequences compared that are not only identical, but also those that have similar properties. Amino acid residues that score a positive value to an amino acid residue of interest are those that are either identical to the amino acid residues of interest or are a preferred substitution (as defined in Table I below) of the amino acid residue of interest.

For purposes herein, the % value of positives of a given amino acid sequence  $A$  to, with, or against a

given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % positives to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

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where X is the number of amino acid residues scoring a positive value as defined above by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acids residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % positives of A to B will not equal the % positives of B to A.

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"Isolated," when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Preferably, the isolated polypeptide is free of association with all components with which it is naturally associated. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the TCCR natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

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An "isolated" nucleic acid molecule encoding a TCCR polypeptide is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the TCCR-encoding nucleic acid. Preferably, the isolated nucleic acid is free of association with all components with which it is naturally associated. An isolated TCCR-encoding nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the TCCR-encoding nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule encoding a TCCR polypeptide includes TCCR-encoding nucleic acid molecules contained in cells that ordinarily express TCCR where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

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The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize, for example, promoters, polyadenylation signals, and enhancers.

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Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in the same

reading frame. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "antibody" is used in the broadest sense and specifically covers, for example, single anti-TCCR monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-TCCR antibody compositions with polypeptidic specificity, single chain anti-TCCR antibodies, and fragments of anti-TCCR antibodies (see below). The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel *et al.*, *Current Protocols in Molecular Biology*, Wiley Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 ug/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

"Moderately stringent conditions" may be identified as described by Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (*e.g.*, temperature, ionic strength and %SDS) less stringent than those described above. In one embodiment, moderately stringent conditions involve overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/mL denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, *etc.* as necessary to accommodate factors such as probe length and the like.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising a polypeptide of the invention fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with the activity of the polypeptide to



which it is fused. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

5 "Active" or "activity" for purposes herein refers to form(s) of proteins of the invention which retain the biologic and/or immunologic activities of a native or naturally-occurring TCCR polypeptide, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring TCCR other than the ability to serve as an antigen in the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring polypeptide of the invention. Similarly, an "immunological" activity refers to the ability to serve as an antigen in the production of an antibody against an antigenic epitope possessed by  
10 a native or naturally-occurring polypeptide of the invention.

"Biological activity" in the context of an antibody or another molecule that can be identified by the screening assays disclosed herein (*e.g.* an organic or inorganic small molecule, peptide, *etc.*) is used to refer to the ability of such molecules to induce or inhibit infiltration of inflammatory cells into a tissue, to stimulate or inhibit T-cell proliferation or activation and to stimulate or inhibit cytokine release by cells. Another preferred activity is increased  
15 vascular permeability or the inhibition thereof. The most preferred activity is the modulation of the Th1/Th2 response (*e.g.*, a decreased Th1 and/or elevated Th2 response, a decreased Th2 and/or elevated Th1 response).

The term "modulation" or "modulating" means the upregulation, downregulation or alteration of the physiology effected by the differentiation of T-cells into the Th1 and Th2 subsets (*e.g.*, cytokine release profiles). Cellular processes within the intended scope of the term may include, but are not limited to: transcription of specific  
20 genes; normal cellular functions, such as metabolism, proliferation, differentiations, adhesion, signal transduction, apoptosis and survival, and abnormal cellular processes such as transformation, blocking of differentiation and metastasis.

The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native sequence TCCR polypeptide of the invention disclosed herein  
25 (*e.g.*, downregulation of a Th1/Th2 cellular function). In a similar manner, the term "agonist" is used in the broadest sense and includes any molecule that mimics, enhances or stimulates a biological activity of a native sequence TCCR polypeptide of the invention disclosed herein. Suitable agonist or antagonist molecules specifically include agonist or antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native polypeptides of the invention, peptides, small organic molecules, *etc.* Methods for identifying agonists or antagonists of a TCCR  
30 polypeptide may comprise contacting a TCCR polypeptide with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with the TCCR polypeptide (*e.g.*, upregulation/downregulation of a Th1/Th2 cellular function or effect).

A "small molecule" is defined herein to have a molecular weight below about 500 daltons, and is generally an organic compound.

35 "Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same general structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas. The term "antibody" is

used in the broadest sense and specifically covers, for example, single anti-TCCR monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies), anti-TCCR antibody compositions with polypeptopic specificity, single chain anti-TCCR antibodies, and fragments of anti-TCCR antibodies (see below). The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. The antibody may bind to any domain of the polypeptide of the invention which may be contacted by the antibody. For example, the antibody may bind to any extracellular domain of the polypeptide and when the entire polypeptide is secreted, to any domain on the polypeptide which is available to the antibody for binding.

"Native antibodies" and "native immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain ( $V_H$ ) followed by a number of constant domains. Each light chain has a variable domain at one end ( $V_L$ ) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light-chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three or four segments called "complementarity-determining regions" (CDRs) or "hypervariable regions" in both the light-chain and the heavy-chain variable domains. There are at least two (2) techniques for determining CDRs: (1) an approach based on cross-species sequence variability (*i.e.*, Kabat *et al.*, *Sequences of Proteins of Immunological Interest* (National Institute of Health, Bethesda, MD 1987); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Chothia, C. *et al.*, *Nature* 342: 877 (1989)). However, to the extent that the two techniques describe different residues they can be combined to define a hybrid CDR.

The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four or five FR regions, largely adopting a  $\beta$ -sheet configuration, connected by the CDRs, which form loops connecting, and in some cases forming part of, the  $\beta$ -sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat *et al.*, NIH Publ. No.91-3242, Vol. I, pages 647-669 (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; diabodies; linear antibodies (Zapata *et al.*, *Protein Eng.* 8(10):1057-1062 [1995]); single-chain antibody molecules; and

multispecific antibodies formed from antibody fragments.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an  $F(ab')_2$  fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the  $V_H$ - $V_L$  dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group.  $F(ab')_2$  antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler *et al.*, *Nature*, 256:495 [1975], or may be made by recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature* 352:624-628 (1991) and

Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), for example. See also U.S. Patent Nos. 5,750,373, 5,571,698, 5,403,484 and 5,223,409 which describe the preparation of antibodies using phagemid and phage vectors.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 [1984]).

"Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues, especially when those particular FR residues impact upon the conformation of the binding site and/or the antibody in three dimensional space. Furthermore, humanized antibodies may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature*, 321:522-525 (1986); Reichmann *et al.*, *Nature*, 332:323-329 [1988]; and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992). Optionally, the humanized antibody may also include a "primatized" antibody where the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest. Antibodies containing residues from Old World monkeys are described, for example, in U.S. Patent Nos. 5,658,570; 5,693,780; 5,681,722; 5,750,105; and 5,756,096.

Antibodies and fragments thereof in this invention also include "affinity matured" antibodies in which an antibody is altered to change the amino acid sequence of one or more of the CDR regions and/or the framework regions to alter the affinity of the antibody or fragment thereof for the antigen to which it binds. Affinity maturation may result in an increase or in a decrease in the affinity of the matured antibody for the antigen relative to the starting antibody. Typically, the starting antibody will be a humanized, human, chimeric or murine antibody and the affinity matured antibody will have a higher affinity than the starting antibody. During the maturation process, one or more of the amino acid residues in the CDRs or in the framework regions are changed to a different residue using any standard method. Suitable methods include point mutations using well known cassette mutagenesis methods (Wells *et al.*, 1985, *Gene* 34:315) or oligonucleotide mediated mutagenesis methods (Zoller *et al.*, 1987, *Nucleic Acids Res.*,

10:6487-6504). Affinity maturation may also be performed using known selection methods in which many mutations are produced and mutants having the desired affinity are selected from a pool or library of mutants based on improved affinity for the antigen or ligand. Known phage display techniques can be conveniently used in this approach. See, for example, U.S. 5,750,373; U.S. 5,223,409, *etc.*

5 Human antibodies are also within the scope of the antibodies of the invention. Human antibodies can be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991); U. S. 5,750, 373].

10 Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific

15 publications: Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14: 845-51 (1996); Neuberger, *Nature Biotechnology* 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

"Single-chain Fv" or "sFv" antibody fragments comprise the V<sub>H</sub> and V<sub>L</sub> domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide

20 linker between the V<sub>H</sub> and V<sub>L</sub> domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V<sub>H</sub>) connected to a light-chain variable domain (V<sub>L</sub>) in the same

25 polypeptide chain (V<sub>H</sub> - V<sub>L</sub>). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993).

The term "isolated" when it refers to the various polypeptides of the invention means a polypeptide which

30 has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the polypeptide of the invention will be purified (1) to greater than 95% by weight of the compound as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain

35 at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated compound, *e.g.* antibody or polypeptide, includes the compound *in situ* within recombinant cells since at least one component of the compound's natural environment will not be present. Ordinarily, however, isolated

compound will be prepared by at least one purification step.

The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the compound, *e.g.* antibody or polypeptide, so as to generate a "labelled" compound. The label may be detectable by itself (*e.g.* radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

By "solid phase" is meant a non-aqueous matrix to which the compound of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.*, controlled pore glass), polysaccharides (*e.g.*, agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phase can comprise the well of an assay plate; in others it is a purification column (*e.g.*, an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Patent No. 4,275,149.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as the anti-ErbB2 antibodies disclosed herein and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (*i.e.*, is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

## **II Compositions and Methods of the Invention**

### **A. Full-length TCCR Polypeptide**

The present invention provides in part a novel method for using TCCR polypeptides to treat immune-related disorders, including the modulation of the differentiation of T-cells into the Th1 and Th2 subtypes and to the treatment of the host of disorders implicated thereby. In particular, cDNAs encoding TCCR polypeptides have been identified, isolated and their use in the treatment of Th1-mediated and Th2-mediated disorders is disclosed in further detail below. It is noted that TCCR defines both the native sequence molecules and variants as provided in the definition section, while the term hTCCR and mTCCR define the singular native sequence polypeptides shown in Figures 3 (SEQ ID NO:1) and 4 (SEQ ID NO:2), respectively. However, for the sake of simplicity, in the present specification the protein encoded by DNA41419 (hTCCR) and/or DNA120632 (mTCCR) as well as all further native homologues and variants included in the foregoing definition of TCCR will be referred to as "TCCR", regardless of their origin or mode of preparation.

The predicted amino acid sequence of the proteins encoded by DNA41419 (hTCCR, SEQ ID NO:1) and DNA120632 (mTCCR, SEQ ID NO:2) can be determined from the nucleotide sequence using routine skill. For the TCCR polypeptide and encoding nucleic acid described herein, Applicants have identified what is believed to be the

reading frame best identifiable with the sequence information available at the time.

Using the ALIGN-2 sequence alignment computer program referenced above, it has been found that the full-length native sequence hTCCR (Figure 3, SEQ ID NO:1) and mTCCR (Figure 4, SEQ ID NO:2) sequence have a certain degree of sequence identity with the Dayhoff (GenBank) sequences having accession numbers 475327 and 7710109.

#### **B. TCCR Variants**

In addition to the full-length native sequence TCCR polypeptides described herein, it is contemplated that TCCR variants can be prepared. TCCR variants can be prepared by introducing appropriate nucleotide changes into the TCCR DNA, and/or by synthesis of the desired TCCR polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of the TCCR, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

Variations in the native full-length sequence TCCR or in various domains of the polypeptide of the TCCR described herein, can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the TCCR that results in a change in the amino acid sequence of the TCCR as compared with the native sequence TCCR. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the TCCR. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the TCCR with that of homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *i.e.*, conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

TCCR polypeptide fragments of the polypeptides of the invention are also within the scope of the invention. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length native protein. Certain fragments lack amino acid residues that are not essential for a desired biological activity of the TCCR polypeptide.

TCCR fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating TCCR fragments by enzymatic digestion, *e.g.*, by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding a desired polypeptide fragment, by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, polypeptide fragments share at least one biological and/or immunological activity with the TCCR polypeptides shown in Figure 3 (SEQ ID NO:1) and Figure 4 (SEQ ID NO:2).

In particular embodiments, conservative substitutions of interest are shown in Table I under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table I, or as further described below in reference to amino acid classes, are introduced and the products screened.

5

**Table I**

	<u>Original Residue</u>	<u>Exemplary Substitutions</u>	<u>Preferred Substitutions</u>
	Ala (A)	val; leu; ile	val
10	Arg (R)	lys; gln; asn	lys
	Asn (N)	gln; his; lys; arg	gln
	Asp (D)	glu	glu
	Cys (C)	ser	ser
	Gln (Q)	asn	asn
15	Glu (E)	asp	asp
	Gly (G)	pro; ala	ala
	His (H)	asn; gln; lys; arg	arg
	Ile (I)	leu; val; met; ala; phe; norleucine	leu
	Leu (L)	norleucine; ile; val; met; ala; phe	ile
20	Lys (K)	arg; gln; asn	arg
	Met (M)	leu; phe; ile	leu
	Phe (F)	leu; val; ile; ala; tyr	leu
	Pro (P)	ala	ala
	Ser (S)	thr	thr
25	Thr (T)	ser	ser
	Trp (W)	tyr; phe	tyr
	Tyr (Y)	trp; phe; thr; ser	phe
	Val (V)	ile; leu; met; phe; ala; norleucine	leu

30 Substantial modifications in function or immunological identity of the invention polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- 35 (1) hydrophobic: norleucine, met, ala, val, leu, ile;  
 (2) neutral hydrophilic: cys, ser, thr;  
 (3) acidic: asp, glu;  
 (4) basic: asn, gln, his, lys, arg;  
 (5) residues that influence chain orientation: gly, pro; and  
 40 (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

45 The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter *et al.*, *Nucl. Acids Res.*, 13:4331 (1986); Zoller *et al.*, *Nucl. Acids Res.*, 10:6487 (1987)], cassette mutagenesis [Wells *et al.*, *Gene*, 34:315



(1985)], restriction selection mutagenesis [Wells *et al.*, *Philos. Trans. R. Soc. London SerA*, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the variant DNA.

Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, *Science*, 244: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions [Creighton, *The Proteins*, (W.H. Freeman & Co., N.Y.); Chothia, *J. Mol. Biol.*, 150:1 (1976)]. If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

### C. Modifications of TCCR

Covalent modifications of TCCR are included within the scope of this invention. One type of covalent modification includes reacting targeted amino acid residues of a TCCR polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of the TCCR. Derivatization with bifunctional agents is useful, for instance, for crosslinking the TCCR to a water-insoluble support matrix or surface for use in the method for purifying anti-TCCR antibodies, and vice-versa. Commonly used crosslinking agents include, *e.g.*, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimide.

Other modifications include deamidation of glutamyl and asparaginy residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the  $\alpha$ -amino groups of lysine, arginine, and histidine side chains [T.E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)], acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

Another type of covalent modification of the invention polypeptide included within the scope of this invention comprises altering the native glycosylation pattern of the polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence polypeptide (either by removing the underlying glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the native sequence. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

Addition of glycosylation sites to the polypeptide may be accomplished by altering the amino acid sequence. The alteration may be made, for example, by the addition of, or substitution by, one or more serine or threonine residues to the native sequence polypeptide (for O-linked glycosylation sites). The amino acid sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

Another means of increasing the number of carbohydrate moieties on the polypeptide of the invention is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, *CRC Crit. Rev. Biochem.*, pp. 259-306 (1981).

Removal of carbohydrate moieties present on the polypeptide of the invention may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, *et al.*, *Arch. Biochem. Biophys.*, 259:52 (1987) and by Edge *et al.*, *Anal. Biochem.*, 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, *Meth. Enzymol.*, 138:350 (1987).

Another type of covalent modification comprises linking the invention polypeptide to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

The TCCR polypeptides of the present invention may also be modified in a way to form a chimeric molecule comprising the invention polypeptide fused to another, heterologous polypeptide or amino acid sequence.

In one embodiment, such a chimeric molecule comprises a fusion of the invention polypeptide with a tag polypeptide which provides an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl- terminus of the polypeptide of the invention. The presence of such epitope-tagged forms of the polypeptide of the invention can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables the polypeptide of the invention to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidinc-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 [Field *et al.*, *Mol. Cell. Biol.*, 8:2159-2165 (1988)]; the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan *et al.*, *Molecular and Cellular Biology*, 5:3610-3616 (1985)]; and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky *et al.*, *Protein Engineering*, 3(6):547-553 (1990)]. Other tag polypeptides include the Flag-peptide [Hopp *et al.*, *BioTechnology*, 6:1204-1210 (1988)]; the KT3 epitope peptide [Martin *et al.*, *Science*, 255:192-194 (1992)]; an  $\alpha$ -tubulin epitope peptide [Skinner *et al.*, *J. Biol. Chem.*, 266:15163-15166 (1991)]; and the T7 gene 10 protein peptide tag [Lutz-Freyermuth *et al.*, *Proc. Natl. Acad. Sci. USA*, 87:6393-6397 (1990)].

In an alternative embodiment, the chimeric molecule may comprise a fusion of the polypeptide of the invention with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of an invention polypeptide in place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130 issued June 27, 1995.

#### **D. Preparation of TCCR**

The description below relates primarily to production of TCCR by culturing cells transformed or

transfected with a vector containing TCCR nucleic acid. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare TCCR. For instance, the TCCR sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques (see, *e.g.*, Stewart *et al.*, Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, *J. Am. Chem. Soc.* 85: 2149-2154 (1963)). *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using the manufacturer's instructions. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of the TCCR may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the full-length TCCR.

### 1. Isolation of DNA Encoding the Polypeptide of the Invention

DNA encoding TCCR may be obtained from a cDNA library prepared from tissue believed to possess the TCCR mRNA and to express it at a detectable level. Accordingly, human TCCR DNA can be conveniently obtained from a cDNA library prepared from human tissue, such as described in the Examples. The TCCR-encoding gene may also be obtained from a genomic library or by oligonucleotide synthesis.

Libraries can be screened with probes (such as antibodies to the polypeptide of the invention or oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press, 1989). An alternative means to isolate the gene encoding the polypeptide of the invention is to use PCR methodology [Sambrook *et al.*, *supra*; Dieffenbach *et al.*, *PCR Primer: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, 1995)].

The Examples below describe techniques for screening a cDNA library. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like <sup>32</sup>P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook *et al.*, *supra*.

Sequences identified in such library screening methods can be compared and aligned to other known sequences deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein.

Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in Sambrook *et al.*, *supra*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

### 2. Selection and Transformation of Host Cells

Host cells are transfected or transformed with expression or cloning vectors described herein for TCCR

production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in

5 *Mammalian Cell Biotechnology: A Practical Approach*, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al.*, *supra*.

Methods of transfection are known to the ordinarily skilled artisan, for example,  $\text{CaCl}_2$ ,  $\text{CaPO}_4$ , liposome-mediated and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook *et al.*, *supra*, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall

10 barriers. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw *et al.*, *Gene*, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, *Virology*, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transformations have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen *et al.*, *J. Bact.*,

15 130:946 (1977) and Hsiao *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown *et al.*, *Methods in Enzymology*, 185:527-537 (1990) and Mansour *et al.*, *Nature*, 336:348-352 (1988).

20 Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli*. Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia*, *e.g.*, *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5

25 772 (ATCC 53,635), *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, *e.g.*, *Salmonella typhimurium*, *Serratia*, *e.g.*, *Serratia marcescans*, and *Shigella*, as well as Bacilli such as *B. subtilis* and *B. licheniformis* (*e.g.*, *B. licheniformis* 41P disclosed in DD266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because

30 it is a common host strain for recombinant NDA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA*; *E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3*; *E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan*;

35 *E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan*; *E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,83 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, *e.g.*, PCR or other nucleic acid polymerase chain reactions, are suitable.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for TCCR encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, *Nature* 290: 140 (1981); EP 139,383 published 2 May 1985); *Kluveromyces* hosts (U.S. Patent No. 4,943,529; Fleer *et al.*, *Bio/Technology* 9: 968-975 (1991)) such as, *e.g.*, *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt *et al.*, *J. Bacteriol.* 154(2): 737 (1983); *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wicheramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilorum* (ATCC 36,906); Van den Berg *et al.*, *Bio/Technology* 8: 135 (1990)), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); Sreekrishna *et al.*, *J. Basic Microbiol.* 28: 265-278 (1988); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case *et al.*, *Proc. Natl. Acad. Sci. USA*, 76:5259-5263 (1979); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, *e.g.*, *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance *et al.*, *Biochem. Biophys. Res. Commun.* 112: 284-289 (1983); Tilburn *et al.*, *Gene* 26: 205-221 (1983); Yelton *et al.*, *Proc. Natl. Acad. Sci. USA* 81: 1470-1474 (1984)) and *A. niger* (Kelly and Hynes, *EMBO J.* 4: 475-479 (1985)). Methylophilic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, *The Biochemistry of Methylophilic* 269 (1982).

Suitable host cells for the expression of glycosylated TCCR polypeptides are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 and high five, as well as plant cells. Examples of useful mammalian host cell lines include Chinese hamster ovary (CHO) and COS cells. More specific examples include monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham *et al.*, *J. Gen Virol.*, 36:59 (1977)); Chinese hamster ovary cells/-DHFR (CHO, Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.*, 23:243-251 (1980)); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); and mouse mammary tumor (MMT 060562, ATCC CCL51). The selection of the appropriate host cell is deemed to be within the skill in the art.

### 3. Selection and Use of a Replicable Vector

The nucleic acid (*e.g.*, cDNA or genomic DNA) encoding TCCR may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, phagemid or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

The TCCR may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector,

or it may be a part of the TCCR-encoding DNA that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, *e.g.*, the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces*  $\alpha$ -factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C. albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2 $\mu$  plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli*.

An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the nucleic acid encoding the polypeptide of the invention, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub *et al.*, *Proc. Natl. Acad. Sci. USA*, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 [Stinchcomb *et al.*, *Nature*, 282:39 (1979); Kingsman *et al.*, *Gene*, 7:141 (1979); Tschemper *et al.*, *Gene*, 10:157 (1980)]. The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, *Genetics*, 85:12 (1977)].

Expression and cloning vectors usually contain a promoter operably linked to the TCCR-encoding nucleic acid sequence to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the  $\beta$ -lactamase and lactose promoter systems [Chang *et al.*, *Nature*, 275:615 (1978); Goeddel *et al.*, *Nature*, 281:544 (1979)], alkaline phosphatase, a tryptophan (*trp*) promoter system [Goeddel, *Nucleic Acids Res.*, 8:4057 (1980); EP 36,776], and hybrid promoters such as the *tac* promoter [deBoer *et al.*, *Proc. Natl. Acad. Sci. USA*, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding TCCR.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman *et al.*, *J. Biol. Chem.*, 255:2073 (1980)] or other glycolytic enzymes [Hess *et al.*, *J. Adv. Enzyme Reg.*, 7:149 (1968); Holland, *Biochemistry*, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription

controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

5 TCCR transcription of the polypeptide of the invention from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters  
10 are compatible with the host cell systems.

Transcription of a DNA encoding the TCCR by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin,  $\alpha$ -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell  
15 virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the TCCR coding sequence of the polypeptide of the invention, but is preferably located at a site 5' from the promoter.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells  
20 from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding TCCR.

Still other methods, vectors, and host cells suitable for adaptation to the synthesis of TCCR in recombinant  
25 vertebrate cell culture are described in Gething *et al.*, *Nature*, 293:620-625 (1981); Mantei *et al.*, *Nature*, 281:40-46 (1979); EP 117,060; and EP 117,058.

#### 4. Detecting Gene Amplification/Expression

Gene amplification and/or expression may be measured in a sample directly, for example, by conventional  
30 Southern blotting, Northern blotting to quantitate the transcription of mRNA [Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 (1980)], dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay may be carried out where the duplex is bound to a surface, so that upon the formation of a duplex on the surface, the presence of antibody bound to the duplex can be detected.

35 Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native

sequence TCCR polypeptide or against a synthetic peptide based on the DNA sequences provided herein or against exogenous sequence fused to TCCR DNA encoding the polypeptide of the invention and encoding a specific antibody epitope.

### 5. Purification of Polypeptide

5 Forms of TCCR may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.* Triton®-X 100) or by enzymatic cleavage. Cells employed in expression of the polypeptide of TCCR can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

10 It may be desired to purify TCCR from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the polypeptide of the invention. Various methods of protein purification may be employed and such methods are known  
15 in the art and described for example in Deutscher, *Methods in Enzymology*, **182** (1990); Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular TCCR produced.

### 6. Tissue Distribution

The location of tissues expressing the polypeptides of the invention can be identified by determining mRNA  
20 expression in various human tissues. The location of such genes provides information about which tissues are most likely to be affected by the stimulating and inhibiting activities of the polypeptides of the invention. The location of a gene in a specific tissue also provides sample tissue for the activity blocking assays discussed below.

As noted before, gene expression in various tissues may be measured by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA*, **77**:5201-5205 [1980]),  
25 dot blotting (DNA analysis), or *in situ* hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes.

Gene expression in various tissues, alternatively, may be measured by immunological methods, such as immunohistochemical staining of tissue sections and assay of cell culture or body fluids, to quantitate directly the  
30 expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies may be prepared against a native sequence of a polypeptide of the invention or against a synthetic peptide based on the DNA sequences encoding the polypeptide of the invention or against an exogenous sequence fused to a DNA encoding a polypeptide of the invention and encoding a specific antibody epitope. General techniques for generating antibodies,  
35 and special protocols for Northern blotting and *in situ* hybridization are provided below.

## E. Uses of TCCR

### 1. General Uses

TCCR is of the WS(G)XWS class of cytokine receptors with homology to the IL-12  $\beta$ -2 receptor, G-



CSFR and IL-6 receptor, the highest homology being to the IL-12  $\beta$ -2 receptor (26% identity). These receptors transduce a signal that can control growth and differentiation of cells, especially cells involved in blood cell growth and differentiation. G-CSF, for example has found wide use in clinical applications for the proliferation of neutrophils after chemotherapy. These types of cytokine receptors and their agonists/antagonists are likely to play important roles in the treatment of hematological and oncological disorders. TCCR has been found to play a role in the T-helper cell response - in particular in the modulation of the differentiation of T-cells into the Th1 and Th2 subsets. As a result, TCCR and its agonists/antagonists may be useful in a therapeutic method to bias the mammalian immune response to either a T-helper 1 response (Th1) or a T-helper-2 (Th2) response depending on the desired therapeutic goal.

CD4+ T cells play a critical role in allergic inflammatory responses by enhancing the recruitment, growth and differentiation of all other cell types involved in the response. CD4+ cells perform this function by secreting several cytokines, including interleukin (IL-4) and IL-13, which enhance the induction of IgE synthesis in B cells, mast cell growth, and the recruitment of lymphocytes, mast cells, and basophils to the sites of inflammation. In addition, CD4+ T cells produce IL-5, which enhances the growth and differentiation of eosinophils and B cells, and IL-10, which enhances the growth and differentiation of mast cells and inhibits the production of  $\gamma$ -interferon. The combination of IL-4, IL-5, IL-10 and IL-13 is produced by a subset of CD4+ T-cells called Th2 cells, which are found in increased abundance in allergic individuals.

Th1 cells secrete cytokines important in the activation of macrophages (IFN- $\gamma$ , IL-2, tumor necrosis factor- $\beta$  [TNF- $\beta$ ]) and in inducing cell mediated immunity. Th2 cells secrete cytokines important in humoral immunity and allergic diseases (IL-4, IL-5 and IL-10). While Th1 cytokines inhibit the production of Th2 cytokines, Th2 cytokines inhibit the production of Th1 cytokines. This negative feedback loop accentuates the production of polarized cytokine profiles during immune responses. The maintenance of the delicate balance between the production of these "opposing" cytokines is critical, since overproduction of Th1 cytokines is believed to result in autoimmune inflammatory diseases and allograft rejection. Concomitantly, the overproduction of Th2 cytokines results in allergic inflammatory diseases such as asthma and allergic rhinitis, or ineffective immunity to intracellular pathogens.

Umetsu and DeKruyff, *Proc. Soc. Exp. Bio. Med.* 215(1): 11-20 (1997) have proposed a model wherein susceptibility to infection is explained not as a lack of immunity, but rather to the development of T cells secreting an in appropriate cytokine profile. Allergic disease is caused by the CD4+ T cells inappropriately secreting Th2 cytokines, whereas nonallergic individuals remain asymptomatic because they develop T cells secreting Th1 cytokines, which inhibit IgE synthesis and mast cell and eosinophil differentiation. Stated another way, allergic rhinitis and asthma may represent a pathological aberration or oral/mucosal tolerance, where T cells that would normally develop into "Th2" regulatory/suppressor cells instead develop into "Th2" cells that initiate and intensify allergic inflammation.

Cytokine receptors are generally characterized by a multi-domain structure comprising an extracellular domain, a transmembrane domain and an intracellular domain. The extracellular domain usually functions to bind the ligand, the transmembrane domain anchors the receptor to the cell membrane, and the intracellular domain is usually an effector involved in signal transduction within the cell. However, ligand-binding and effector functions may reside on separate subunits of a multimeric receptor. The ligand-binding domain may itself have multiple domains.

Multimeric receptors is a broad term which generally includes: (1) homodimer; (2) heterodimers having subunits with both ligand-binding and effector domains; and (3) multimers having component subunits with disparate functions. Cytokine receptors are further reviewed and classified in Urdahl, *Ann. Reports Med. Chem.* 26: 221-228 (1991) and Cosman, *Cytokine* 5: 95-106 (1993).

5           In addition to specific immune-related uses (*e.g.*, Th1 and Th2 cells mediated physiology), nucleotide sequences (or their complement) encoding TCCR have various applications in the art of molecular biology, including uses as hybridization probes, in chromosome and gene mapping and in the generation of anti-sense RNA and DNA. TCCR nucleic acid will also be useful for the preparation of TCCR polypeptides by the recombinant techniques described herein.

10           The full-length native sequence TCCR gene described in Figure 3 (SEQ ID NO:1) and Figure 4 (SEQ ID NO:2), or portions thereof, may be used as hybridization probes for a cDNA library to isolate the full-length TCCR cDNA or to isolate still other cDNAs (for instance, those encoding naturally-occurring variants of TCCR or TCCR from other species) which have a desired sequence identity to the TCCR sequence disclosed in Figures 3 and 4 (SEQ ID NO:s 1&2, respectively). Optionally, the length of the probes will be about 20 to 50 bases. The hybridization  
15 probes may be derived from regions of the nucleotide sequence of SEQ ID NO:1&2 wherein those regions may be determined without undue experimentation or from genomic sequences including promoters, enhancer elements and introns of native sequence TCCR. By way of example, a screening method will comprise isolating the coding region of the TCCR gene using the known DNA sequence to synthesize a selected probe of about 40 bases. Hybridization probes may be labeled by a variety of labels, including radionucleotides such as <sup>32</sup>P or <sup>35</sup>S, or enzymatic labels such  
20 as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems. Labeled probes having a sequence complementary to that of the TCCR gene of the present invention can be used to screen libraries of human cDNA, genomic DNA or mRNA to determine to which members of such libraries the probe hybridizes. Hybridization techniques are described in further detail in the Examples below. Any EST or other sequence fragments disclosed herein may similarly be employed as probes, using the methods disclosed herein.

25           Other useful fragments of the TCCR nucleic acids include antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target TCCR mRNA (sense) or TCCR DNA (antisense) sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of TCCR DNA. Such a fragment generally comprises at least about 14 nucleotides, preferably from about 14 to 30 nucleotides. The ability to derive an antisense or a sense oligonucleotide, based upon  
30 a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48: 2659 (1988) and van der Krol *et al.*, *BioTechniques* 6: 958 (1988).

          Binding of antisense or sense oligonucleotides to target nucleic acid sequences results in the formation of duplexes that block transcription or translation of the target sequence by one of several means, including enhanced degradation of the duplexes, premature termination of transcription or translation, or by other means. The antisense  
35 oligonucleotides thus may be used to block expression of TCCR proteins. Antisense or sense oligonucleotides further comprise oligonucleotides having modified sugar-phosphodiester backbones (or other sugar linkages, such as those described in WO 91/06629) and wherein such sugar linkages are resistant to endogenous nucleases. Such oligonucleotides with resistant sugar linkages are stable *in vivo* (*i.e.*, capable of resisting enzymatic digestion) but

retain sequence specificity to be able to bind to target nucleotide sequences.

Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10048, and other moieties that increase affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotides to modify binding specificities for the antisense or sense oligonucleotide for the target nucleotide sequence.

Antisense or sense oligonucleotides may be introduced into a cell containing the target nucleic acid sequence by any gene transfer method, including, for example,  $\text{CaPO}_4$ -mediated DNA transfection, electroporation, or by using gene transfer vectors such as Epstein-Barr virus. In a preferred procedure, an antisense or sense oligonucleotide is inserted into a suitable retroviral vector. A cell containing the target nucleic acid sequence is contacted with the recombinant retroviral vector, either *in vivo* or *ex vivo*. Suitable retroviral vectors include, but are not limited to, those derived from the murine retrovirus M-MuLV, N2 (a retrovirus derived from M-MuLV), or the double copy vectors designated DCT5A, DCT5B and DCT5C (see WO 90/13641).

Sense or antisense oligonucleotides also may be introduced into a cell containing the target nucleotide sequence by formation of a conjugate with a ligand binding molecule, as described in WO 91/04753. Suitable ligand binding molecules include, but are not limited to, cell surface receptors, growth factors, other cytokines, or other ligands that bind to cell surface receptors. Preferably, conjugation of the ligand binding molecule does not substantially interfere with the ability of the ligand binding molecule to bind to its corresponding molecule or receptor, or block entry of the sense or antisense oligonucleotide or its conjugated version into the cell.

Alternatively, a sense or an antisense oligonucleotide may be introduced into a cell containing the target nucleic acid sequence by formation of an oligonucleotide-lipid complex, as described in WO 90/10448. The sense or antisense oligonucleotide-lipid complex is preferably dissociated within the cell by an endogenous lipase.

The probes may also be employed in PCR techniques to generate a pool of sequences for identification of closely related TCCR coding sequences.

Nucleotide sequences encoding a TCCR can also be used to construct hybridization probes for mapping the gene which encodes that TCCR and for the genetic analysis of individuals with genetic disorders. The nucleotide sequences provided herein may be mapped to a chromosome and specific regions of a chromosome using known techniques, such as *in situ* hybridization, linkage analysis against known chromosomal markers, and hybridization screening with libraries.

Since TCCR is a receptor, the coding sequences for TCCR encode a protein which binds to another protein. As a result, the TCCR proteins of the invention can be used in assays to identify other proteins or molecules involved in the binding interaction. By such methods, inhibitors of the receptor/ligand binding interaction can be identified. Proteins involved in such binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction. Also, the receptor TCCR can be used to isolate correlative ligand(s). Screening assays can be used to find lead compounds that mimic the biological activity of a native TCCR or a ligand for TCCR. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated

include synthetic organic or inorganic compounds. The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art.

5 The TCCR polypeptides described herein may also be employed as molecular weight markers for protein electrophoresis purposes.

The nucleic acid molecules encoding the TCCR polypeptides or fragments thereof described herein are useful for chromosome identification. In this regard, there exists an ongoing need to identify new chromosome markers, since relatively few chromosome marking reagents, based upon actual sequence data are presently available. Each TCCR nucleic acid molecule of the present invention can be used as a chromosome marker.

10 The TCCR polypeptides and nucleic acid molecules of the present invention may also be used for tissue typing, wherein the TCCR polypeptides of the present invention may be differentially expressed in one tissue as compared to another. TCCR nucleic acid molecules will find use for generating probes for PCR, Northern analysis, Southern analysis and Western analysis.

## 2. Antibody Binding Studies

15 The activity of the TCCR polypeptides of the invention can be further verified by antibody binding studies, in which the ability of anti-TCCR antibodies to inhibit the effect of the TCCR polypeptides on tissue cells is tested. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies, the preparation of which will be described hereinbelow.

20 Antibody binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Zola, *Monoclonal Antibodies: A Manual of Techniques*, pp.147-158 (CRC Press, Inc., 1987).

25 Competitive binding assays rely on the ability of a labeled standard to compete with the test sample analyte for binding with a limited amount of antibody. The amount of target protein in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies preferably are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies may conveniently be separated from the standard and analyte which remain unbound.

30 Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the protein to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex. See, e.g., US Pat No. 4,376,110. The second antibody may itself be labeled with a detectable moiety (direct sandwich assays) or may be measured using an anti-immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

35 For immunohistochemistry, the tissue sample may be fresh or frozen or may be embedded in paraffin and fixed with a preservative such as formalin, for example.

## 3. Cell-Based Assays

Cell-based assays and animal models for immune related diseases can be used to further understand the

relationship between the genes and polypeptides identified herein and the development and pathogenesis of immune related disease.

In a different approach, cells of a cell type known to be involved in a particular immune related disease are transfected with the cDNAs described herein, and the ability of these cDNAs to stimulate or inhibit immune function is analyzed. Suitable cells can be transfected with the desired gene, and monitored for immune function activity. Such transfected cell lines can then be used to test the ability of poly- or monoclonal antibodies or antibody compositions to inhibit or stimulate immune function, for example to modulate T-cell proliferation or inflammatory cell infiltration. Cells transfected with the coding sequences of the genes identified herein can further be used to identify drug candidates for the treatment of immune related diseases.

In addition, primary cultures derived from transgenic animals (as described below) can be used in the cell-based assays herein, although stable cell lines are preferred. Techniques to derive continuous cell lines from transgenic animals are well known in the art (see, e.g. Small *et al.*, *Mol. Cell. Biol.* 5, 642-648 [1985]).

One suitable cell based assay is the mixed lymphocyte reaction (MLR). *Current Protocols in Immunology*, unit 3.12; edited by J E Coligan, A M Kruisbeek, D H Marglies, E M Shevach, W Strober, National Institutes of Health, Published by John Wiley & Sons, Inc. In this assay, the ability of a test compound to stimulate or inhibit the proliferation of activated T cells is assayed. A suspension of responder T cells is cultured with allogeneic stimulator cells and the proliferation of T cells is measured by uptake of tritiated thymidine. This assay is a general measure of T cell reactivity. Since the majority of T cells respond to and produce IL-2 upon activation, differences in responsiveness in this assay in part reflect differences in IL-2 production by the responding cells. The MLR results can be verified by a standard lymphokine (IL-2) detection assay. *Current Protocols in Immunology*, above, 3.15, 6.3.

A proliferative T cell response in an MLR assay may be due to direct mitogenic properties of an assayed molecule or to external antigen induced activation. Additional verification of the T cell stimulatory activity of the polypeptides of the invention can be obtained by a costimulation assay. T cell activation requires an antigen specific signal mediated through the T-cell receptor (TCR) and a costimulatory signal mediated through a second ligand binding interaction, for example, the B7(CD80, CD86)/CD28 binding interaction. CD28 crosslinking increases lymphokine secretion by activated T cells. T cell activation has both negative and positive controls through the binding of ligands which have a negative or positive effect. CD28 and CTLA-4 are related glycoproteins in the Ig superfamily which bind to B7. CD28 binding to B7 has a positive costimulation effect of T cell activation; conversely, CTLA-4 binding to B7 has a negative T cell deactivating effect. Chambers, C. A. and Allison, J. P., *Curr. Opin. Immunol.* (1997) 9:396. Schwartz, R. H., *Cell* (1992) 71:1065; Linsey, P. S. and Ledbetter, J. A., *Annu. Rev. Immunol.* (1993) 11:191; June, C. H. *et al.*, *Immunol. Today* (1994) 15:321; Jenkins, M. K., *Immunity* (1994) 1:405. In a costimulation assay, the polypeptides of the invention are assayed for T cell costimulatory or inhibitory activity.

Polypeptides of the invention, as well as other compounds of the invention, which are stimulators (costimulators) of T cell proliferation and agonists, e.g. agonist antibodies, thereto as determined by MLR and costimulation assays, for example, are useful in treating immune related diseases characterized by poor, suboptimal or inadequate immune function. These diseases are treated by stimulating the proliferation and activation of T cells (e.g., T cell mediated immunity, Th1 and/or Th2 cytokine production) and enhancing the immune response in a

mammal through administration of a stimulatory compound, such as the stimulating polypeptides of the invention. The stimulating polypeptide may, for example, be a TCCR ligand polypeptide or an agonist antibody thereof.

Direct use of a stimulating compound as in the invention has been validated in experiments with 4-1BB glycoprotein, a member of the tumor necrosis factor receptor family, which binds to a ligand (4-1BBL) expressed on primed T cells and signals T cell activation and growth. Alderson, M. E. *et al.*, *J. Immunol.* (1994) 24:2219.

The use of an agonist stimulating compound has also been validated experimentally. Activation of 4-1BB by treatment with an agonist anti-4-1BB antibody enhances eradication of tumors. Hellstrom, I. and Hellstrom, K. E., *Crit. Rev. Immunol.* (1998) 18:1. Immunoadjuvant therapy for treatment of tumors, described in more detail below, is another example of the use of the stimulating compounds of the invention.

An immune stimulating or enhancing effect can also be achieved by antagonizing or blocking the activity of a protein which has been found to be inhibiting in the MLR assay. Negating the inhibitory activity of the compound produces a net stimulatory effect. Suitable antagonists/blocking compounds are antibodies or fragments thereof which recognize and bind to the inhibitory protein, thereby blocking the effective interaction of the protein with its receptor and inhibiting signaling through the receptor. This effect has been validated in experiments using anti-CTLA-4 antibodies which enhance T cell proliferation, presumably by removal of the inhibitory signal caused by CTLA-4 binding. Walunas, T. L. *et al.*, *Immunity* (1994) 1:405.

On the other hand, polypeptides of the invention, as well as other compounds of the invention, which are direct inhibitors of T cell proliferation/activation and/or lymphokine secretion, can be directly used to suppress the immune response. These compounds are useful to reduce the degree of the immune response and to treat immune related diseases characterized by a hyperactive, superoptimal, or autoimmune response. This use of the compounds of the invention may be validated by the experiments described above in which CTLA-4 binding to receptor B7 deactivates T cells. The direct inhibitory compounds of the invention function in an analogous manner.

Alternatively, compounds, *e.g.* antibodies, which bind to stimulating polypeptides of the invention and block the stimulating effect of these molecules produce a net inhibitory effect and can be used to suppress the T cell mediated immune response by inhibiting T cell proliferation/activation and/or lymphokine secretion. Blocking the stimulating effect of the polypeptides suppresses the immune response of the mammal. This use has been validated in experiments using an anti-IL2 antibody. In these experiments, the antibody binds to IL2 and blocks binding of IL2 to its receptor thereby achieving a T cell inhibitory effect.

#### 4. Animal Models

The results of the cell based *in vitro* assays can be further verified using *in vivo* animal models and assays for T-cell function. A variety of well known animal models can be used to further understand the role of the genes identified herein in the development and pathogenesis of immune related disease, and to test the efficacy of candidate therapeutic agents, including antibodies, and other antagonists of the native polypeptides, including small molecule antagonists. The *in vivo* nature of such models makes them predictive of responses in human patients. Animal models of immune related diseases include both non-recombinant and recombinant (transgenic) animals. Non-recombinant animal models include, for example, rodent, *e.g.*, murine models. Such models can be generated by introducing cells into syngeneic mice using standard techniques, *e.g.* subcutaneous injection, tail vein injection, spleen implantation, intraperitoneal implantation, implantation under the renal capsule, *etc.*

Graft-versus-host disease occurs when immunocompetent cells are transplanted into immunosuppressed or tolerant patients. The donor cells recognize and respond to host antigens. The response can vary from life threatening severe inflammation to mild cases of diarrhea and weight loss. Graft-versus-host disease models provide a means of assessing T cell reactivity against MHC antigens and minor transplant antigens. A suitable procedure is described in detail in *Current Protocols in Immunology*, above, unit 4.3.

An animal model for skin allograft rejection is a means of testing the ability of T cells to mediate *in vivo* tissue destruction and a measure of their role in transplant rejection. The most common and accepted models use murine tail-skin grafts. Repeated experiments have shown that skin allograft rejection is mediated by T cells, helper T cells and killer-effector T cells, and not antibodies. Auchincloss, H. Jr. and Sachs, D. H., *Fundamental Immunology*, 2nd ed., W. E. Paul ed., Raven Press, NY, 1989, 889-992. A suitable procedure is described in detail in *Current Protocols in Immunology*, above, unit 4.4. Other transplant rejection models which can be used to test the compounds of the invention are the allogeneic heart transplant models described by Tanabe, M. *et al*, *Transplantation* (1994) 58:23 and Tinubu, S. A. *et al*, *J. Immunol.* (1994) 4330-4338.

Animal models for delayed type hypersensitivity provides an assay of cell mediated immune function as well. Delayed type hypersensitivity reactions are a T cell mediated *in vivo* immune response characterized by inflammation which does not reach a peak until after a period of time has elapsed after challenge with an antigen. These reactions also occur in tissue specific autoimmune diseases such as multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE, a model for MS). A suitable procedure is described in detail in *Current Protocols in Immunology*, above, unit 4.5.

EAE is a T cell mediated autoimmune disease characterized by T cell and mononuclear cell inflammation and subsequent demyelination of axons in the central nervous system. EAE is generally considered to be a relevant animal model for MS in humans. Bolton, C., *Multiple Sclerosis* (1995) 1:143. Both acute and relapsing-remitting models have been developed. The compounds of the invention can be tested for T cell stimulatory or inhibitory activity against immune mediated demyelinating disease using the protocol described in *Current Protocols in Immunology*, above, units 15.1 and 15.2. See also the models for myelin disease in which oligodendrocytes or Schwann cells are grafted into the central nervous system as described in Duncan, I. D. *et al*, *Molec. Med. Today* (1997) 554-561.

Contact hypersensitivity is a simple delayed type hypersensitivity *in vivo* assay of cell mediated immune function. In this procedure, cutaneous exposure to exogenous haptens which gives rise to a delayed type hypersensitivity reaction which is measured and quantitated. Contact sensitivity involves an initial sensitizing phase followed by an elicitation phase. The elicitation phase occurs when the T lymphocytes encounter an antigen to which they have had previous contact. Swelling and inflammation occur, making this an excellent model of human allergic contact dermatitis. A suitable procedure is described in detail in *Current Protocols in Immunology*, Eds. J. E. Cologan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach and W. Strober, John Wiley & Sons, Inc., 1994, unit 4.2. See also Grabbe, S. and Schwarz, T, *Immun. Today* 19(1):37-44 (1998) .

An animal model for arthritis is collagen-induced arthritis. This model shares clinical, histological and immunological characteristics of human autoimmune rheumatoid arthritis and is an acceptable model for human autoimmune arthritis. Mouse and rat models are characterized by synovitis, erosion of cartilage and subchondral bone. The compounds of the invention can be tested for activity against autoimmune arthritis using the protocols described

in *Current Protocols in Immunology*, above, units 15.5. See also the model using a monoclonal antibody to CD18 and VLA-4 integrins described in Issekutz, A. C. *et al.*, *Immunology* (1996) 88:569.

A model of asthma has been described in which antigen-induced airway hyper-reactivity, pulmonary eosinophilia and inflammation are induced by sensitizing an animal with ovalbumin and then challenging the animal with the same protein delivered by aerosol. Several animal models (guinea pig, rat, non-human primate) show symptoms similar to atopic asthma in humans upon challenge with aerosol antigens. Murine models have many of the features of human asthma. Suitable procedures to test the compounds of the invention for activity and effectiveness in the treatment of asthma are described by Wolyniec, W. W. *et al.*, *Am. J. Respir. Cell Mol. Biol.* (1998) 18:777 and the references cited therein.

Additionally, the compounds of the invention can be tested on animal models for psoriasis like diseases. Evidence suggests a T cell pathogenesis for psoriasis. The compounds of the invention can be tested in the scid/scid mouse model described by Schon, M. P. *et al.*, *Nat. Med.* (1997) 3:183, in which the mice demonstrate histopathologic skin lesions resembling psoriasis. Another suitable model is the human skin/scid mouse chimera prepared as described by Nickoloff, B. J. *et al.*, *Am. J. Pathol.* (1995) 146:580.

Recombinant (transgenic) animal models can be engineered by introducing the coding portion of the genes identified herein into the genome of animals of interest, using standard techniques for producing transgenic animals. Animals that can serve as a target for transgenic manipulation include, without limitation, mice, rats, rabbits, guinea pigs, sheep, goats, pigs, and non-human primates, *e.g.* baboons, chimpanzees and monkeys. Techniques known in the art to introduce a transgene into such animals include pronucleic microinjection (Hoppe and Wanger, U.S. Patent No. 4,873,191); retrovirus-mediated gene transfer into germ lines (*e.g.*, Van der Putten *et al.*, *Proc. Natl. Acad. Sci. USA* 82: 6148-615 [1985]); gene targeting in embryonic stem cells (Thompson *et al.*, *Cell* 56: 313-321 [1989]); electroporation of embryos (Lo, *Mol. Cell. Biol.* 3, 1803-1814 [1983]); sperm-mediated gene transfer (Lavitrano *et al.*, *Cell* 57, 717-73 [1989]). For review, see, for example, U.S. Patent No. 4,736,866.

For the purpose of the present invention, transgenic animals include those that carry the transgene only in part of their cells ("mosaic animals"). The transgene can be integrated either as a single transgene, or in concatamers, *e.g.*, head-to-head or head-to-tail tandems. Selective introduction of a transgene into a particular cell type is also possible by following, for example, the technique of Lasko *et al.*, *Proc. Natl. Acad. Sci. USA* 89, 6232-636 (1992).

The expression of the transgene in transgenic animals can be monitored by standard techniques. For example, Southern blot analysis or PCR amplification can be used to verify the integration of the transgene. The level of mRNA expression can then be analyzed using techniques such as *in situ* hybridization, Northern blot analysis, PCR, or immunocytochemistry.

The animals may be further examined for signs of immune disease pathology, for example by histological examination to determine infiltration of immune cells into specific tissues. Blocking experiments can also be performed in which the transgenic animals are treated with the compounds of the invention to determine the extent of the T cell proliferation stimulation or inhibition of the compounds. In these experiments, blocking antibodies which bind to the polypeptide of the invention, prepared as described above, are administered to the animal and the effect on immune function is determined.

Nucleic acids which encode TCCR or its modified forms can also be used to generate either transgenic



animals or "knock out" animals which, in turn, are useful in the development and screening of therapeutically useful reagents. The term "knockout" is used in the art to describe a transgenic animal in which the endogenous gene has been "knocked out" or ablated such as that which results from the use of homologous recombination. Homologous recombination is a term of art used to describe the regions of the targeting vector that are homologous to the endogenous gene. These regions of homology will hybridize to each other and recombine to the host's genome resulting with the replacement of the host endogenous sequence with the vector insert sequence at the location and in the orientation defined by the regions of shared homology. The genotype of a knockout animal is denoted by the name of the gene followed by a "-/-". This distinguishes it from an animal in which only one allele has been "knocked-out" (heterozygous) which is termed "-/+". An endogenous gene that has been "knocked out" is no longer expressed in all cells throughout the animal. Detailed analysis of specific cells can identify the function of the ablated gene.

A transgenic animal (*e.g.*, a mouse or rat) is an animal having cells that contain a transgene, which transgene was introduced into the animal or an ancestor of the animal at a prenatal, *e.g.*, an embryonic stage. A transgene is a DNA which is integrated into the genome of a cell from which a transgenic animal develops. In one embodiment, cDNA encoding TCCR can be used to clone genomic DNA encoding TCCR in accordance with established techniques and the genomic sequences used to generate transgenic animals that contain cells which express DNA encoding TCCR. Methods for generating transgenic animals, particularly animals such as mice or rats, have become conventional in the art and are described, for example, in U.S. Patent Nos. 4,736,866 and 4,870,009. Typically, particular cells would be targeted for TCCR transgene incorporation with tissue-specific enhancers. Transgenic animals that include a copy of a transgene encoding TCCR introduced into the germ line of the animals at an embryonic stage can be used to examine the effect of increased expression of DNA encoding TCCR. Such animals can be used as tester animals for reagents thought to confer protection from, for example, pathological conditions associated with its overexpression. In accordance with this facet of the invention, an animal is treated with the reagent and a reduced incidence of the pathological condition, compared to untreated animals bearing the transgene, would indicate a potential therapeutic intervention for the pathological condition.

Alternatively, "knock out" animals can be constructed which have a defective or altered gene encoding a polypeptide identified herein, as a result of homologous recombination between the endogenous gene encoding the polypeptide and altered genomic DNA encoding the same polypeptide introduced into an embryonic cell of the animal. For example, cDNA encoding a particular polypeptide can be used to clone genomic DNA encoding that polypeptide in accordance with established techniques. A portion of the genomic DNA encoding a particular polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see *e.g.*, Thomas and Capecchi, *Cell*, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (*e.g.*, by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see *e.g.*, Li *et al.*, *Cell*, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (*e.g.*, a mouse or rat) to form aggregation chimeras [see *e.g.*, Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny

harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the polypeptide.

5 For the present invention, knockout mice were created in order to study the effect of TCCR agonization/antagonization of the Th1 and/or Th2 immune response and disorders mediated thereby.

#### 5. Chimeric receptors

Additionally, chimeric receptors can be recreated to determine the effect of signaling by a receptor having an unknown ligand. Chimeric receptors are a proven means of examining the function of a receptor's function  
10 without isolation of the ligand. Chang *et al.*, *Mol. Cell Biol.* 18(2): 896-905 (1998).

#### 6. ImmunoAdjuvant Therapy

In one embodiment, the immunostimulating compounds of the invention can be used in immunoadjuvant therapy for the treatment of tumors (cancer). It is now well established that T cells recognize human tumor specific antigens. One group of tumor antigens, encoded by the MAGE, BAGE and GAGE families of genes, are silent in all  
15 adult normal tissues, but are expressed in significant amounts in tumors, such as melanomas, lung tumors, head and neck tumors, and bladder carcinomas. DeSmet, C. *et al.*, (1996) *Proc. Natl. Acad. Sci. USA*, 93:7149. It has been shown that costimulation of T cells induces tumor regression and an antitumor response both *in vitro* and *in vivo*. Melero, I. *et al.*, *Nature Medicine* (1997) 3:682; Kwon, E. D. *et al.*, *Proc. Natl. Acad. Sci. USA* (1997) 94:8099; Lynch, D. H. *et al.*, *Nature Medicine* (1997) 3:625; Finn, O. J. and Lotze, M. T., *J. Immunol.* (1998) 21:114. The stimulatory  
20 compounds of the invention can be administered as adjuvants, alone or together with a growth regulating agent, cytotoxic agent or chemotherapeutic agent, to stimulate T cell proliferation/activation and an antitumor response to tumor antigens. The growth regulating, cytotoxic, or chemotherapeutic agent may be administered in conventional amounts using known administration regimes. Immunostimulating activity by the compounds of the invention allows reduced amounts of the growth regulating, cytotoxic, or chemotherapeutic agents thereby potentially lowering  
25 the toxicity to the patient.

#### 7. Screening Assays for Drug Candidates

Screening assays for drug candidates are designed to identify compounds that bind to or complex with the polypeptides encoded by the TCCR nucleic acids identified herein or a biologically active variant thereof, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening  
30 assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds, including peptides, preferably soluble peptides, (poly)peptide-immunoglobulin fusions, and, in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single-chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as  
35 well as human antibodies and antibody fragments.

The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays and cell based assays, which are well characterized in the art. All of the drug candidate screening assays identified herein have the property in common that they call for contacting the drug

candidate with an TCCR polypeptide under conditions and for a time sufficient to allow these two molecules to interact.

In binding assays, the interaction is binding and the complex formed can be isolated or detected in the reaction mixture. Since the TCCR polypeptides of the present invention are receptors, a TCCR ECD fragment may also be suitably employed for the purpose of identifying drug candidates including TCCR variants, antagonists thereof and/or agonists thereof. In a particular embodiment, the polypeptide encoded by the gene identified herein or the drug candidate is immobilized on a solid phase, *e.g.* on a microtiter plate, by covalent or non-covalent attachments. Non-covalent attachment generally is accomplished by coating the solid surface with a solution of the polypeptide and drying. Alternatively, an immobilized antibody, *e.g.* a monoclonal antibody, specific for the polypeptide to be immobilized can be used to anchor it to a solid surface. The assay is performed by adding the non-immobilized component, which may be labeled by a detectable label, to the immobilized component, *e.g.* the coated surface containing the anchored component. When the reaction is complete, the non-reacted components are removed, *e.g.* by washing, and complexes anchored on the solid surface are detected. When the originally non-immobilized component carries a detectable label, the detection of label immobilized on the surface indicates that complexing has occurred. Where the originally non-immobilized component does not carry a label, complexing can be detected, for example, by using a labelled antibody specifically binding the immobilized complex.

If the candidate compound interacts with but does not bind to a particular TCCR protein identified herein, its interaction with that protein can be assayed by methods well known for detecting protein-protein interactions. Such assays include traditional approaches, such as, cross-linking, co-immunoprecipitation, and co-purification through gradients or chromatographic columns. In addition, protein-protein interactions can be monitored by using a yeast-based genetic system described by Fields and co-workers [Fields and Song, *Nature (London)* **340**, 245-246 (1989); Chien *et al.*, *Proc. Natl. Acad. Sci. USA* **88**: 9578-9582 (1991)] as disclosed by Chevray and Nathans [*Proc. Natl. Acad. Sci. USA* **89**: 5789-5793 (1991)]. Many transcriptional activators, such as yeast GAL4, consist of two physically discrete modular domains, one acting as the DNA-binding domain, while the other one functioning as the transcription activation domain. The yeast expression system described in the foregoing publications (generally referred to as the "two-hybrid system") takes advantage of this property, and employs two hybrid proteins, one in which the target protein is fused to the DNA-binding domain of GAL4, and another, in which candidate activating proteins are fused to the activation domain. The expression of a GAL1-*lacZ* reporter gene under control of a GAL4-activated promoter depends on reconstitution of GAL4 activity via protein-protein interaction. Colonies containing interacting polypeptides are detected with a chromogenic substrate for  $\beta$ -galactosidase. A complete kit (MATCHMAKER™) for identifying protein-protein interactions between two specific proteins using the two-hybrid technique is commercially available from Clontech. This system can also be extended to map protein domains involved in specific protein interactions as well as to pinpoint amino acid residues that are crucial for these interactions.

In order to find compounds that interfere with the interaction of a TCCR polypeptide identified herein and other intra- or extracellular components can be tested, a reaction mixture is usually prepared containing the product of the gene and the intra- or extracellular component under conditions and for a time allowing for the interaction and binding of the components. To test the ability of a test compound to inhibit the above interactions, the reaction is run

in the absence and in the presence of the test compound. In addition, a placebo may be added to a third reaction mixture, to serve as a positive control. The binding (complex formation) between the test compound and the intra- or extracellular component present in the mixture is monitored as described above. The formation of a complex in the control reaction(s) but not in the reaction mixture containing the test compound indicates that the test compound interferes with the interaction of the test compound and its reaction partner.

#### 8. Compositions and Methods for the Treatment of Immune Related Diseases

The compositions useful in the treatment of immune related diseases (*e.g.*, Th1- and/or Th2-mediated disorders) include, without limitation, proteins, antibodies, small organic molecules, peptides, phosphopeptides, antisense and ribozyme molecules, triple helix molecules, *etc.* that inhibit or stimulate immune function, for example, T cell proliferation/activation, lymphokine release, or immune cell infiltration.

For example, antisense RNA and RNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. When antisense DNA is used, oligodeoxyribonucleotides derived from the translation initiation site, *e.g.* between about -10 and +10 positions of the target gene nucleotide sequence, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details see, *e.g.* Rossi, *Current Biology* 4: 469-471 (1994), and PCT publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details see, *e.g.* PCT publication No. WO 97/33551, *supra*.

These molecules can be identified by any or any combination of the screening assays discussed above and/or by any other screening techniques well known for those skilled in the art.

The TCCR polypeptides, agonists and antagonists (TCCR molecules) described herein may also be employed as therapeutic agents. The TCCR molecules of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the TCCR molecule is combined in combination with a pharmaceutically acceptable carrier vehicle. Therapeutic formulations are prepared for storage by mixing the TCCR molecules having the desired degree of purity with optional physiologically acceptable carriers, excipients or stabilizers, *Remington's Pharmaceutical Sciences* 16th edition. Osol. A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN®, PLURONICS® or PEG.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution.

Therapeutic compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having as stopper pierceable by a hypodermic injection needle.

5 The route of administration is in accord with known methods, *e.g.*, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial or intralesional routes, topical administration, or by sustained release systems.

Dosages and desired drug concentrations of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary physician. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The use of interspecies scaling in toxicokinetics" in *Toxicokinetics and New Drug Development*, Yacobi *et al.*, Eds., Pergamon Press, New York 1989, pp. 42-96.

When *in vivo* administration of a TCCR molecules thereof is employed, normal dosage amounts may vary from about 10 ng/kg to up to 100 mg/kg of mammal body weight or more per day, preferably about 1 µg/kg/day to 10 mg/kg/day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760; 5,206,344 or 5,225,212. It is anticipated that different formulations will be effective for different treatments and different disorders, and that administration intended to treat a specific organ or tissue, may necessitate delivery in a manner different from that to another organ or tissue.

Where sustained-release administration of TCCR molecules is desired in a formulation with release characteristics suitable for the treatment of any disease or disorder requiring administration of the TCCR molecules, microencapsulation of the TCCR molecules is contemplated. Microencapsulation of recombinant proteins for sustained release has been successfully performed with human growth hormone (rhGH), interferon-α, -β, -γ (rhIFN-α, -β, -γ), interleukin-2, and MN rgp120. Johnson *et al.*, *Nat. Med.* 2: 795-799 (1996); Yasuda, *Biomed. Ther.* 27: 1221-1223 (1993); Hora *et al.*, *Bio/Technology* 8: 755-758 (1990); Cleland, "Design and Production of Single Immunization Vaccines Using Polylactide Polyglycolide Microsphere Systems" in *Vaccine Design: The Subunit and Adjuvant Approach*, Powell and Newman, eds., (Plenum Press: New York, 1995), pp. 439-462; WO 97/03692, WO 96/40072, WO 96/07399 and U.S. Pat. No. 5,654,010.

30 The sustained-release formulations of TCCR molecules may be developed using poly-lactic-coglycolic acid (PLGA), a polymer exhibiting a strong degree of biocompatibility and a wide range of biodegradable properties. The degradation products of PLGA, lactic and glycolic acids, are cleared quickly from the human body. Moreover, the degradability of this polymer can be adjusted from months to years depending on its molecular weight and composition. For further information see Lewis, "Controlled Release of Bioactive Agents from Lactide/Glycolide polymer," in *Biodegradable Polymers as Drug Delivery Systems* M. Chasin and R. Langeer, editors (Marcel Dekker: New York, 1990), pp. 1-41.

## 9. Identification of Agonists and Antagonists of TCCR

The present invention also provides for methods of screening compounds to identify those that mimic or

enhances a TCCR polypeptide effect (agonists) or prevent or inhibit one or more functions or activities of an TCCR polypeptide. Preferably such antagonists and agonists are TCCR variants, peptide fragments small molecules, antisense oligonucleotides (DNA or RNA) or antibodies (monoclonal, humanized, specific, single-chain, heteroconjugate or fragment of the aforementioned). Additionally, TCCR antagonists can include potential TCCR ligands, while  
5 potential TCCR agonists can include soluble TCCR extracellular domains (ECD).

Screening assays for antagonist and/or agonist drug candidates are designed to identify compounds that bind or complex with the TCCR polypeptides encoded by the genes identified herein, or otherwise interfere with the interaction of the encoded polypeptides with other cellular proteins. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them particularly suitable for identifying small  
10 molecule drug candidates.

The assays can be performed in a variety of formats, including protein-protein binding assays, biochemical screening assays, immunoassays, and cell-based assays, which are well characterized in the art.

The screening assays contemplated herein for antagonists have in common the process of contacting the drug candidate with a TCCR polypeptide under conditions and for a time sufficient to allow these two components to  
15 interact.

Examples of suitable assays useful to identify TCCR antagonists and agonists have been identified previously above under 7. *Screening Assays for Drug Candidates*.

As an additional example of an antagonists assay, the TCCR polypeptide may be added to a cell along with the compound to be screened for a particular activity and the ability of the compound to inhibit the activity of interest  
20 in the presence of the TCCR polypeptide indicates that the compound is an antagonist to the TCCR polypeptide. Alternatively, antagonists may be detected by combining the TCCR polypeptide and a potential antagonist with membrane-bound TCCR polypeptide receptors or recombinant receptors under appropriate conditions for a competitive inhibition assay. The TCCR polypeptide can be labeled, such as by radioactivity, such that the number of TCCR polypeptide molecules bound to the receptor can be used to determine the effectiveness of the potential  
25 antagonist. The gene encoding the receptor can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting. Coligan *et al.*, *Current Protocols in Immunol.* 1(2): Ch 5 (1991). Preferably, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the TCCR polypeptide and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the TCCR polypeptide. Transfected cells that are grown on glass slides are  
30 exposed to labeled TCCR polypeptide. The TCCR polypeptide can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase. Following fixation and incubation, the slides are subjected to autoradiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an interactive sub-pooling and re-screening process, eventually yielding a single clone that encodes the putative receptor.

35 In another assay for antagonists, mammalian cells or a membrane preparation expressing the receptor would be incubated with labeled TCCR polypeptide in the presence of the candidate compound. The ability of the compound to enhance or block this interaction could then be measured.

More specific examples of potential antagonists include an oligonucleotide that binds to the fusions of

immunoglobulin with TCCR polypeptide, and in particular, antibodies including, without limitation, poly- and monoclonal antibodies and antibody fragments, single chain antibodies, anti-idiotypic antibodies, and chimeric or humanized versions of such antibodies or fragments, as well as human antibodies and antibody fragments. Alternatively, a potential antagonist may be a closely related protein, for example, a mutated form of the TCCR polypeptide that recognized the ligand but imparts no effect, thereby competitively inhibiting the action of the TCCR polypeptide. Finally, another potential TCCR antagonist is a TCCR ECD which can compete for available ligand, effectively leaving the native TCCR receptor signal free.

Another potential TCCR polypeptide antagonist is an antisense RNA or DNA construct prepared using antisense technology, where, *e.g.*, an antisense RNA or DNA molecule acts to block directly the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA.

For example, the 5' coding portion of the polynucleotide sequence, which encodes the mature TCCR polypeptides herein, is used to design an antisense RNA oligonucleotide from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al.*, *Nucl. Acids. Res.* **6**: 3073 (1979); Cooney *et al.*, *Science* **241**: 456 (1988); Dervan *et al.*, *Science*, **251**: 1360 (1991)), thereby preventing transcription and the production of the TCCR polypeptide. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into the TCCR polypeptide (antisense - Okano, *Nerochem.* **56**: 560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression (CRC Press: Boca Raton, FL, 1988). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of the TCCR polypeptide. When antisense DNA is used, oligodeoxyribonucleotides derived from the translation-initiation site, *e.g.*, between about -10 and +10 positions of the target gene nucleotide sequence are preferred.

Potential antagonists include small molecules that bind to the active site, the receptor binding site, or growth factor or other relevant binding site of the TCCR polypeptide, thereby blocking the normal biological activity of the TCCR polypeptide. Examples of small molecules include, but are not limited to, small peptides or peptide-like molecules, preferably soluble peptides, and synthetic non-peptidyl organic or inorganic compounds.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. Ribozymes act by sequence-specific hybridization to the complementary target RNA, followed by endonucleolytic cleavage. Specific ribozyme cleavage sites within a potential RNA target can be identified by known techniques. For further details, see, *e.g.* Rossi, *Current Biology*, **4**: 469-471 (1994), and PCR publication No. WO 97/33551 (published September 18, 1997).

Nucleic acid molecules in triple-helix formation used to inhibit transcription should be single-stranded and composed of deoxynucleotides. The base composition of these oligonucleotides is designed such that it promotes triple-helix formation via Hoogsteen base-pairing rules, which generally require sizeable stretches of purines or pyrimidines on one strand of a duplex. For further details, see, *e.g.*, PCT publication No. WO 97/33551, *supra*.

These molecules can be identified by any one or more of the screening assays used hereinabove and/or by any other screening techniques well known for those skilled in the art.

## 10. TCCR and gene therapy

Nucleic acid encoding the TCCR polypeptides may also be used in gene therapy. In gene therapy applications, genes are introduced into cells in order to achieve *in vivo* synthesis of a therapeutically effective genetic product, for example for replacement of a defective gene. "Gene therapy" includes both conventional gene therapy where a lasting effect is achieved by a single treatment, and the administration of gene therapeutic agents, which involves the one time or repeated administration of a therapeutically effective amount of DNA or mRNA. Antisense RNAs and DNAs can be used as therapeutic agents for blocking the expression of certain genes *in vivo*. It has already been shown that short antisense oligonucleotides can be imported into cells where they act as inhibitors, despite their low intracellular concentrations caused by their restricted uptake by the cell membrane. Zamecnik *et al.*, *Proc. Natl. Acad. Sci. USA* 83: 4143-4146 (1986)). The oligonucleotides can be modified to enhance their uptake, *e.g.*, by substituting their negatively charged phosphodiester groups by uncharged groups.

There are a variety of techniques available for introducing nucleic acids into viable cells. The techniques vary depending upon whether the nucleic acid is transferred into cultured cells *in vitro*, or *in vivo* in the cells of the intended host. Techniques suitable for the transfer of nucleic acid into mammalian cells *in vitro* include the use of liposomes, electroporation, microinjection, cell fusion, DEAE-dextran, the calcium phosphate precipitation method, *etc.* The currently preferred *in vivo* gene transfer techniques include transfection with viral (typically retroviral) vectors and viral coat protein-liposome mediated transfection (Dzau *et al.*, *Trends in Biotechnology* 11: 205-210 (1993)). In some situations it is desirable to provide the nucleic acid source with an agent that targets the target cells, such as an antibody specific for a cell surface membrane protein or the target cell, a ligand for a receptor on the target cell, *etc.* Where liposomes are employed, proteins which bind to a cell surface membrane protein associated with endocytosis may be used for targeting and/or to facilitate uptake, *e.g.*, capsid proteins or fragments thereof tropic for a particular cell type, antibodies for proteins which undergo internalization in cycling, proteins that target intracellular localization and enhance intracellular half-life. The technique of receptor-mediated endocytosis is described, for example, by Wu *et al.*, *J. Bio. Chem.* 262: 4429-4432 (1987); and Wagner *et al.*, *Proc. Natl. Acad. Sci. USA* 87: 3410-3414 (1990). For review of gene marking and gene therapy protocols see Anderson *et al.*, *Science* 256: 808-813 (1992).

## 11. Antibodies

The present invention further provides anti-TCCR antibodies. Exemplary antibodies include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies, including antibody fragments which may inhibit (antagonists) or stimulate (agonists) T cell proliferation, eosinophil infiltration, *etc.*

### i. Polyclonal Antibodies

The anti-TCCR antibodies may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the TCCR polypeptide or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin



inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

ii. Monoclonal Antibodies

5           The anti-TCCR antibodies may, alternatively, be monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

10           The immunizing agent will typically include the TCCR polypeptide or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell [Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are  
15 usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT  
20 medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Rockville, Maryland.  
25 Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, Marcel Dekker, Inc., New York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against TCCR. Preferably, the binding specificity of monoclonal antibodies produced  
30 by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, *Anal. Biochem.* 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures  
35 and grown by standard methods [Goding, *supra*]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium

or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxyapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison *et al.*, *supra*] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

*In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art.

### **iii. Human and Humanized Antibodies**

The anti-TCCR antibodies of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and coworkers [Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeven *et al.*, *Science*, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole *et al.* and Boerner *et al.* are also available for the preparation of human monoclonal antibodies (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991); U. S. 5,750, 373]. Similarly, human antibodies can be made by introducing of human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks *et al.*, *Bio/Technology* 10, 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-13 (1994); Fishwild *et al.*, *Nature Biotechnology* 14: 845-51 (1996); Neuberger, *Nature Biotechnology* 14: 826 (1996); Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

The antibodies may also be affinity matured using known selection and/or mutagenesis methods as described above. Preferred affinity matured antibodies have an affinity which is five times, more preferably 10 times, even more preferably 20 or 30 times greater than the starting antibody (generally murine, humanized or human) from which the matured antibody is prepared.

#### iv. Bispecific Antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities may be for the polypeptide of the invention, the other one is for any other antigen, and preferably for a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the coexpression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, *Nature*, 305:537-539 [1983]). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Trautnecker *et al.*, *EMBO J.*, 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are cotransfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, *Methods in Enzymology*, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains form the interface of the first antibody molecule are replaced with larger side chains (*e.g.*, tryosine or tryptophan). Compensatory "cavities" of identical or similar size to the large chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with small ones (*e.g.*, alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.*, F(ab')<sub>2</sub> bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan *et al.*, *Science* 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')<sub>2</sub> fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby *et al.*, *J. Exp. Med.* 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')<sub>2</sub> molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques are known for making and isolating bispecific antibody fragments directly from recombinant cell culture. For example, bispecific antibodies have been produced using leucine zippers. Kostelny *et al.*, *J. Immunol.* 148(5): 1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993) has provided as alternative mechanism for making bispecific antibody fragments. The

fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruger *et al.*, *J. Immunol.* 152:5368 (1994). Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt *et al.*, *J. Immunol.* 147: 60 (1991).

Exemplary bispecific antibodies may bind to two different epitopes on a given TCCR polypeptide. Alternatively, an anti-TCCR polypeptide arm may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.*, CD2, CD3, CD28 or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular TCCR polypeptide. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular TCCR polypeptide. These antibodies possess a TCCR-binding arm and an arm which binds a cytotoxic agent or a radionucleotide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the TCCR polypeptide and further binds tissue factor (TF).

v. **Heteroconjugate Antibodies**

Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells [U.S. Patent No. 4,676,980], and for treatment of HIV infection [WO 91/00360; WO 92/200373; EP 03089]. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

vi. **Effector function engineering**

It may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance the effectiveness of the antibody in treating an immune related disease, for example. For example cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron *et al.*, *J. Exp Med.* 176:1191-1195 (1992) and Shopes, B. *J. Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff *et al.* *Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson *et al.*, *Anti-Cancer Drug Design* 3:219-230 (1989).

vii. **Immunoconjugates**

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.* an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include  $^{212}\text{Bi}$ ,  $^{131}\text{I}$ ,  $^{131}\text{In}$ ,  $^{90}\text{Y}$  and  $^{186}\text{Re}$ .

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta *et al.*, *Science* 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tissue pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide).

#### **viii. Immunoliposomes**

The proteins, antibodies, *etc.* disclosed herein may also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein *et al.*, *Proc. Natl. Acad. Sci. USA* 82:3688 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA* 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin *et al.*, *J. Biol. Chem.* 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent (such as doxorubicin) may be optionally contained within the liposome. See Gabizon *et al.*, *J. National Cancer Inst.* 81(19):1484 (1989).

#### **ix. Uses for anti-TCCR Antibodies**

The anti-TCCR antibodies of the present invention have various utilities. For example, anti-TCCR antibodies may be used in diagnostic assays for TCCR, e.g., detecting its expression in specific cells, tissues, or serum. Various diagnostic assay techniques known in the art may be used, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogenous phases [Zola, *Monoclonal Antibodies: A Manual of Techniques*, CRC Press, Inc. (1987) pp. 147-158]. The antibodies used in the diagnostic assays can be labeled with a detectable moiety. The detectable moiety should be capable of

producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$  or  $^{125}\text{I}$ , a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter *et al.*, *Nature* **144**: 945 (1962); David *et al.*, *Biochemistry* **13**: 1014 (1974); Pain *et al.*, *J. Immunol. Meth.* **40**: 219 (1981) and Nygren, *J. Histochem. Cytochem.* **30**: 407 (1982).

Anti-TCCR antibodies also are useful for the affinity purification of TCCR from recombinant cell culture or natural sources. In this process, the antibodies against TCCR are immobilized on a suitable support, such as a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the TCCR to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except the TCCR, which is bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the TCCR from the antibody.

#### 10. Pharmaceutical Compositions

The active molecules of the invention, polypeptides and antibodies, as well as other molecules identified by the screening assays disclosed above, can be administered for the treatment of immune related diseases, in the form of pharmaceutical compositions.

In order to target the intracellular portion of TCCR or to target TCCR while it is still intracellular, internalizing antibodies may be used. Additionally, lipofections or liposomes can also be used to deliver the antibody, or an antibody fragment, into cells. Where antibody fragments are used, the smallest inhibitory fragment that specifically binds to the binding domain of the target protein is preferred. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. See, *e.g.*, Marasco *et al.*, *Proc. Natl. Acad. Sci. USA* **90**: 7889-7893 (1993).

Therapeutic formulations of the active molecule, preferably a polypeptide or antibody of the invention, are prepared for storage by mixing the active molecule having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as TWEEN<sup>TM</sup>, PLURONICS<sup>TM</sup> or polyethylene glycol (PEG).

Compounds identified by the screening assays of the present invention can be formulated in an analogous

manner, using standard techniques well known in the art.

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or growth inhibitory agent.

5 Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

The active molecules may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are  
10 disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of  
15 shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and methyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl  
20 acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated antibodies remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37°C, resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S-S bond formation through thio-disulfide  
25 interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

## 11. Methods of Treatment

It is contemplated that the polypeptides, antibodies and other active compounds of the present invention may be used to treat various immune related diseases and conditions, such as T cell mediated diseases, including  
30 those characterized by infiltration of inflammatory cells into a tissue, stimulation of T-cell proliferation, inhibition of T-cell proliferation, increased or decreased vascular permeability or the inhibition thereof.

Exemplary conditions or disorders to be treated with the polypeptides, antibodies and other compounds of the invention, include, but are not limited to systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, osteoarthritis, spondyloarthropathies, systemic sclerosis (scleroderma), idiopathic inflammatory myopathies  
35 (dermatomyositis, polymyositis), Sjögren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease



(glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, 5 granulomatous hepatitis, and sclerosing cholangitis, inflammatory bowel disease (ulcerative colitis: Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases 10 including graft rejection and graft -versus-host-disease.

In systemic lupus erythematosus, the central mediator of disease is the production of auto-reactive antibodies to self proteins/tissues and the subsequent generation of immune-mediated inflammation. antibodies either directly or indirectly mediate tissue injury. Though T lymphocytes have not been shown to be directly involved in tissue damage, T lymphocytes are required for the development of auto-reactive antibodies. The genesis of the disease is 15 thus T lymphocyte dependent. Multiple organs and systems are affected clinically including kidney, lung, musculoskeletal system, mucocutaneous, eye, central nervous system, cardiovascular system, gastrointestinal tract, bone marrow and blood.

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that mainly involves the synovial membrane of multiple joints with resultant injury to the articular cartilage. The pathogenesis is T lymphocyte 20 dependent and is associated with the production of rheumatoid factors, auto-antibodies directed against self IgG, with the resultant formation of immune complexes that attain high levels in joint fluid and blood. These complexes in the joint may induce the marked infiltrate of lymphocytes and monocytes into the synovium and subsequent marked synovial changes; the joint space/fluid is infiltrated by similar cells with the addition of numerous neutrophils. Tissues affected are primarily the joints, often in symmetrical pattern. However, extra-articular disease also occurs 25 in two major forms. One form is the development of extra-articular lesions with ongoing progressive joint disease and typical lesions of pulmonary fibrosis, vasculitis, and cutaneous ulcers. The second form of extra-articular disease is the so called Felty's syndrome which occurs late in the RA disease course, sometimes after joint disease has become quiescent, and involves the presence of neutropenia, thrombocytopenia and splenomegaly. This can be accompanied by vasculitis in multiple organs with formations of infarcts, skin ulcers and gangrene. Patients often also develop 30 rheumatoid nodules in the subcutis tissue overlying affected joints; the nodules late stage have necrotic centers surrounded by a mixed inflammatory cell infiltrate. Other manifestations which can occur in RA include: pericarditis, pleuritis, coronary arteritis, interstitial pneumonitis with pulmonary fibrosis, keratoconjunctivitis sicca, and rheumatoid nodules.

Juvenile chronic arthritis is a chronic idiopathic inflammatory disease which begins often at less than 16 35 years of age. Its phenotype has some similarities to RA; some patients which are rheumatoid factor positive are classified as juvenile rheumatoid arthritis. The disease is sub-classified into three major categories: pauciarticular, polyarticular, and systemic. The arthritis can be severe and is typically destructive and leads to joint ankylosis and retarded growth. Other manifestations can include chronic anterior uveitis and systemic amyloidosis.

Spondyloarthropathies are a group of disorders with some common clinical features and the common association with the expression of HLA-B27 gene product. The disorders include: ankylosing spondylitis, Reiter's syndrome (reactive arthritis), arthritis associated with inflammatory bowel disease, spondylitis associated with psoriasis, juvenile onset spondyloarthropathy and undifferentiated spondyloarthropathy. Distinguishing features include sacroileitis with or without spondylitis; inflammatory asymmetric arthritis; association with HLA-B27 (a serologically defined allele of the HLA-B locus of class I MHC); ocular inflammation, and absence of autoantibodies associated with other rheumatoid disease. The cell most implicated as key to induction of the disease is the CD8+ T lymphocyte, a cell which targets antigen presented by class I MHC molecules. CD8+ T cells may react against the class I MHC allele HLA-B27 as if it were a foreign peptide expressed by MHC class I molecules. It has been hypothesized that an epitope of HLA-B27 may mimic a bacterial or other microbial antigenic epitope and thus induce a CD8+ T cells response.

Systemic sclerosis (scleroderma) has an unknown etiology. A hallmark of the disease is induration of the skin; likely this is induced by an active inflammatory process. Scleroderma can be localized or systemic; vascular lesions are common and endothelial cell injury in the microvasculature is an early and important event in the development of systemic sclerosis; the vascular injury may be immune mediated. An immunologic basis is implied by the presence of mononuclear cell infiltrates in the cutaneous lesions and the presence of anti-nuclear antibodies in many patients. ICAM-1 is often upregulated on the cell surface of fibroblasts in skin lesions suggesting that T cell interaction with these cells may have a role in the pathogenesis of the disease. Other organs involved include: the gastrointestinal tract: smooth muscle atrophy and fibrosis resulting in abnormal peristalsis/motility; kidney: concentric subendothelial intimal proliferation affecting small arcuate and interlobular arteries with resultant reduced renal cortical blood flow, results in proteinuria, azotemia and hypertension; skeletal muscle: atrophy, interstitial fibrosis; inflammation; lung: interstitial pneumonitis and interstitial fibrosis; and heart: contraction band necrosis, scarring/fibrosis.

Idiopathic inflammatory myopathies including dermatomyositis, polymyositis and others are disorders of chronic muscle inflammation of unknown etiology resulting in muscle weakness. Muscle injury/inflammation is often symmetric and progressive. Autoantibodies are associated with most forms. These myositis-specific autoantibodies are directed against and inhibit the function of components, proteins and RNA's, involved in protein synthesis.

Sjögren's syndrome is due to immune-mediated inflammation and subsequent functional destruction of the tear glands and salivary glands. The disease can be associated with or accompanied by inflammatory connective tissue diseases. The disease is associated with autoantibody production against Ro and La antigens, both of which are small RNA-protein complexes. Lesions result in keratoconjunctivitis sicca, xerostomia, with other manifestations or associations including biliary cirrhosis, peripheral or sensory neuropathy, and palpable purpura.

Systemic vasculitis are diseases in which the primary lesion is inflammation and subsequent damage to blood vessels which results in ischemia/necrosis/degeneration to tissues supplied by the affected vessels and eventual end-organ dysfunction in some cases. Vasculitides can also occur as a secondary lesion or sequelae to other immune-inflammatory mediated diseases such as rheumatoid arthritis, systemic sclerosis, *etc.*, particularly in diseases also associated with the formation of immune complexes. Diseases in the primary systemic vasculitis group include: systemic necrotizing vasculitis: polyarteritis nodosa, allergic angiitis and granulomatosis, polyangiitis; Wegener's granulomatosis; lymphomatoid granulomatosis; and giant cell arteritis. Miscellaneous vasculitides include:

mucocutaneous lymph node syndrome (MLNS or Kawasaki's disease), isolated CNS vasculitis, Behet's disease, thromboangiitis obliterans (Buerger's disease) and cutaneous necrotizing venulitis. The pathogenic mechanism of most of the types of vasculitis listed is believed to be primarily due to the deposition of immunoglobulin complexes in the vessel wall and subsequent induction of an inflammatory response either via ADCC, complement activation, or both.

Sarcoidosis is a condition of unknown etiology which is characterized by the presence of epithelioid granulomas in nearly any tissue in the body; involvement of the lung is most common. The pathogenesis involves the persistence of activated macrophages and lymphoid cells at sites of the disease with subsequent chronic sequelae resultant from the release of locally and systemically active products released by these cell types.

Autoimmune hemolytic anemia including autoimmune hemolytic anemia, immune pancytopenia, and paroxysmal nocturnal hemoglobinuria is a result of production of antibodies that react with antigens expressed on the surface of red blood cells (and in some cases other blood cells including platelets as well) and is a reflection of the removal of those antibody coated cells via complement mediated lysis and/or ADCC/Fc-receptor-mediated mechanisms.

In autoimmune thrombocytopenia including thrombocytopenic purpura, and immune-mediated thrombocytopenia in other clinical settings, platelet destruction/removal occurs as a result of either antibody or complement attaching to platelets and subsequent removal by complement lysis, ADCC or FC-receptor mediated mechanisms.

Thyroiditis including Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, and atrophic thyroiditis, are the result of an autoimmune response against thyroid antigens with production of antibodies that react with proteins present in and often specific for the thyroid gland. Experimental models exist including spontaneous models: rats (BUF and BB rats) and chickens (obese chicken strain); inducible models: immunization of animals with either thyroglobulin, thyroid microsomal antigen (thyroid peroxidase).

Type I diabetes mellitus or insulin-dependent diabetes is the autoimmune destruction of pancreatic islet  $\beta$  cells; this destruction is mediated by auto-antibodies and auto-reactive T cells. Antibodies to insulin or the insulin receptor can also produce the phenotype of insulin-non-responsiveness.

Immune mediated renal diseases, including glomerulonephritis and tubulointerstitial nephritis, are the result of antibody or T lymphocyte mediated injury to renal tissue either directly as a result of the production of autoreactive antibodies or T cells against renal antigens or indirectly as a result of the deposition of antibodies and/or immune complexes in the kidney that are reactive against other, non-renal antigens. Thus other immune-mediated diseases that result in the formation of immune-complexes can also induce immune mediated renal disease as an indirect sequelae. Both direct and indirect immune mechanisms result in inflammatory response that produces/induces lesion development in renal tissues with resultant organ function impairment and in some cases progression to renal failure. Both humoral and cellular immune mechanisms can be involved in the pathogenesis of lesions.

Demyelinating diseases of the central and peripheral nervous systems, including multiple sclerosis; idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome; and Chronic Inflammatory Demyelinating Polyneuropathy, are believed to have an autoimmune basis and result in nerve demyelination as a result of damage caused to oligodendrocytes or to myelin directly. In MS there is evidence to suggest that disease induction and progression is

dependent on T lymphocytes. Multiple Sclerosis is a demyelinating disease that is T lymphocyte-dependent and has either a relapsing-remitting course or a chronic progressive course. The etiology is unknown; however, viral infections, genetic predisposition, environment, and autoimmunity all contribute. Lesions contain infiltrates of predominantly T lymphocyte mediated, microglial cells and infiltrating macrophages; CD4+T lymphocytes are the predominant cell type at lesions. The mechanism of oligodendrocyte cell death and subsequent demyelination is not known but is likely T lymphocyte driven.

Inflammatory and Fibrotic Lung Disease, including Eosinophilic Pneumonias; Idiopathic Pulmonary Fibrosis, and Hypersensitivity Pneumonitis may involve a dysregulated immune-inflammatory response. Inhibition of that response would be of therapeutic benefit.

Autoimmune or Immune-mediated Skin Disease including Bullous Skin Diseases, Erythema Multiforme, and Contact Dermatitis are mediated by auto-antibodies, the genesis of which is T lymphocyte-dependent.

Psoriasis is a T lymphocyte-mediated inflammatory disease. Lesions contain infiltrates of T lymphocytes, macrophages and antigen processing cells, and some neutrophils.

Allergic diseases, including asthma; allergic rhinitis; atopic dermatitis; food hypersensitivity; and urticaria are T lymphocyte dependent. These diseases are predominantly mediated by T lymphocyte induced inflammation, IgE mediated-inflammation or a combination of both.

Transplantation associated diseases, including Graft rejection and Graft-Versus-Host-Disease (GVHD) are T lymphocyte-dependent; inhibition of T lymphocyte function is ameliorative.

Other diseases in which intervention of the immune and/or inflammatory response have benefit are infectious disease including but not limited to viral infection (including but not limited to AIDS, hepatitis A, B, C, D, E and herpes) bacterial infection, fungal infections, and protozoal and parasitic infections (molecules (or derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the immune response to infectious agents), diseases of immunodeficiency (molecules/derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the immune response for conditions of inherited, acquired, infectious induced (as in HIV infection), or iatrogenic (*i.e.* as from chemotherapy) immunodeficiency, and neoplasia.

It has been demonstrated that some human cancer patients develop an antibody and/or T lymphocyte response to antigens on neoplastic cells. It has also been shown in animal models of neoplasia that enhancement of the immune response can result in rejection or regression of that particular neoplasm. Molecules that enhance the T lymphocyte response in the MLR have utility *in vivo* in enhancing the immune response against neoplasia. Molecules which enhance the T lymphocyte proliferative response in the MLR (or small molecule agonists or antibodies that affect the same receptor in an agonistic fashion) can be used therapeutically to treat cancer. Molecules that inhibit the lymphocyte response in the MLR also function *in vivo* during neoplasia to suppress the immune response to a neoplasm; such molecules can either be expressed by the neoplastic cells themselves or their expression can be induced by the neoplasm in other cells. Antagonism of such inhibitory molecules (either with antibody, small molecule antagonists or other means) enhances immune-mediated tumor rejection.

Additionally, inhibition of molecules with proinflammatory properties may have therapeutic benefit in reperfusion injury; stroke; myocardial infarction; atherosclerosis; acute lung injury; hemorrhagic shock; burn; sepsis/septic shock; acute tubular necrosis; endometriosis; degenerative joint disease and pancreatitis.

The compounds of the present invention, *e.g.* polypeptides or antibodies, are administered to a mammal, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation (intranasal, intrapulmonary) routes. Intravenous or inhaled administration of polypeptides and antibodies is preferred.

In immunoadjuvant therapy, other therapeutic regimens, such administration of an anti-cancer agent, may be combined with the administration of the proteins, antibodies or compounds of the instant invention. For example, the patient to be treated with an immunoadjuvant of the invention may also receive an anti-cancer agent (chemotherapeutic agent) or radiation therapy. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *Chemotherapy Service* Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992). The chemotherapeutic agent may precede, or follow administration of the immunoadjuvant or may be given simultaneously therewith. Additionally, an anti-oestrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see, EP 616812) may be given in dosages known for such molecules.

It may be desirable to also administer antibodies against other immune disease associated or tumor associated antigens, such as antibodies which bind to CD20, CD11a, CD18, ErbB2, EGFR, ErbB3, ErbB4, or vascular endothelial factor (VEGF). Alternatively, or in addition, two or more antibodies binding the same or two or more different antigens disclosed herein may be coadministered to the patient. Sometimes, it may be beneficial to also administer one or more cytokines to the patient. In one embodiment, the polypeptides of the invention are coadministered with a growth inhibitory agent. For example, the growth inhibitory agent may be administered first, followed by a polypeptide of the invention. However, simultaneous administration or administration first is also contemplated. Suitable dosages for the growth inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth inhibitory agent and the polypeptide of the invention.

For the treatment or reduction in the severity of immune related disease, the appropriate dosage of an a compound of the invention will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the compound, and the discretion of the attending physician. The compound is suitably administered to the patient at one time or over a series of treatments.

For example, depending on the type and severity of the disease, about 1  $\mu\text{g/kg}$  to 15  $\text{mg/kg}$  (*e.g.* 0.1-20 $\text{mg/kg}$ ) of polypeptide or antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1  $\mu\text{g/kg}$  to 100  $\text{mg/kg}$  or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

## 12. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the

diagnosis or treatment of the disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for diagnosing or treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agent in the composition is usually a polypeptide or an antibody of the invention. The label on, or associated with, the container indicates that the composition is used for diagnosing or treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

### 13. Diagnosis and Prognosis of Immune Related Disease

Cell surface proteins, such as proteins which are overexpressed in certain immune related diseases, are excellent targets for drug candidates or disease treatment. The same proteins along with secreted proteins encoded by the genes amplified in immune related disease states find additional use in the diagnosis and prognosis of these diseases. For example, antibodies directed against the protein products of genes amplified in multiple sclerosis, rheumatoid arthritis, or another immune related disease, can be used as diagnostics or prognostics.

For example, antibodies, including antibody fragments, can be used to qualitatively or quantitatively detect the expression of proteins encoded by amplified or overexpressed genes ("marker gene products"). The antibody preferably is equipped with a detectable, *e.g.* fluorescent label, and binding can be monitored by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. These techniques are particularly suitable, if the overexpressed gene encodes a cell surface protein. Such binding assays are performed essentially as described above.

*In situ* detection of antibody binding to the marker gene products can be performed, for example, by immunofluorescence or immunoelectron microscopy. For this purpose, a histological specimen is removed from the patient, and a labeled antibody is applied to it, preferably by overlaying the antibody on a biological sample. This procedure also allows for determining the distribution of the marker gene product in the tissue examined. It will be apparent for those skilled in the art that a wide variety of histological methods are readily available for *in situ* detection.

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

### EXAMPLES

Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA. Unless otherwise noted, the present invention uses standard procedures of recombinant DNA technology, such as

those described hereinabove and in the following textbooks: Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press N.Y., 1989; Ausubel *et al.*, *Current Protocols in Molecular Biology*, Green Publishing Associates and Wiley Interscience, N.Y., 1989; Innis *et al.*, *PCR Protocols: A Guide to Methods and Applications*, Academic Press, inc., N.Y., 1990; Harlow *et al.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, 1988; Gait, M.J., *Oligonucleotide Synthesis*, IRL Press, Oxford, 1984; R.I. Freshney, *Animal Cell Culture*, 1987; Coligan *et al.*, *Current Protocols in Immunology*, 1991.

### **EXAMPLE 1**

#### **Isolation and cloning of TCCR**

Cytokine receptors and/or receptor characterized by a WS(G)XWS domain were used to search public EST databases and resulted in the isolation of hTCCR (SEQ ID NO:1) and mTCCR (mTCCR).

Alternatively, the murine TCCR depicted in Figure 4 (SEQ ID NO:2) has been published in WO97/44455 filed on 23 May 1996 as well as in GenBank as accession number 7710109. The prior art molecule is also described in Sprecher *et al.*, *Biochem. Biophys. Res. Commun.* 246(1): 82-90 (1998). In Figure 4 (SEQ ID NO:2), a signal peptide has been identified from amino acid residues 1 to about 24, the transmembrane domain from about amino acid residues 514 to about 532, N-glycosylation sites at about residues, 46-49, 296-299, 305-308, 360-361, 368-371 and 461-464, casein kinase II phosphorylation sites at about residues 10-13, 93-96, 130-133, 172-175, 184-187, 235-238, 271-274, 272-275, 323-326, 606-609 and 615-618, a tyrosine kinase phosphorylation site at about residues 202-209, N-myristoylation sites at about residues 43-48, 102-107, 295-300, 321-326, 330-335, 367-342, 393-398, 525-530 and 527-532, an amidation site at about residues 240-243, a prokaryotic membrane lipoprotein lipid attachment at about residues 516-526 and a growth factor and cytokine receptor family signature 1 at about residues 36-49. Region of significant homology exist with: (1) human erythropoietin at about residues 14-51 and (2) murine interleukin-5 receptor at residues 211-219.

A polypeptide having high homology to the human TCCR depicted in Figure 3 (SEQ ID NO:1) has been published in WO 97/44455 filed on 23 May 1996 which is also available from GenBank as accession number 4759327. The prior art molecule is also described in Sprecher *et al.*, *Biochem. Biophys. Res. Commun.* 246(1): 82-90 (1998). In Figure 3 (SEQ ID NO:1), a signal peptide has been identified from amino acid residues 1 to about 32, the transmembrane domain from about amino acid residues 517 to about 538, N-glycosylation sites at about residues 51-54, 76-79, 302-305, 311-314, 374-377, 382-385, 467-470, 563-566, N-myristoylation sites at about residues 107-112, 240-245, 244-249, 281-286, 292-297, 373-378, 400-405, 459-464, 470-475, 531-536 and 533-538, a prokaryotic membrane lipoprotein lipid attachment site at about residues 522-532 and a growth factor and cytokine receptor family signature 1 at about residues 41-54. There is also a region of significant homology with the second subunit of the receptor for human granulocyte-macrophage colony-stimulating factor (GM-CSF) at residues 183-191.

A comparison of the human TCCR (SEQ ID NO:1) and murine TCCR (SEQ ID NO:2) sequences is shown in Figure 5. The comparison reveals about 62% sequence identity between the human and the murine sequences.

### **EXAMPLE 2**

#### **TCCR "knockout" mice**

##### **1. Preparation of the targeting vector**

The term "targeting vector" is a term of art referring to a nucleic acid sequence that is constructed for gene

ablation. Figure 9A describes the targeting vector used for the TCCR molecule isolated in this example. Specifically, the targeting vector was constructed using a 2.4 kb XhoI-HindIII fragment containing the first two exons and a 6.0 kb EcoRI-BamHI fragment containing exons 9 through 14. The specific TCCR gene isolated contains 14 exons and 13 introns. In this targeting vector, the pGK-neo gene conferring gentamycin resistance has been used to replace exons 3-8, leaving exons 1 and 2 intact. The herpes simplex virus thymidine kinase (HSV-TK) coding region has been placed 5' of exon one, allowing for selection with gancyclovir. Such drug selectable makers, such as gancyclovir permit for selection of stable transfected cell lines containing the targeting vector and further allow for polymerase chain reaction (PCR) primers to be made which will amplify a fragment of nucleic acid unique to the targeting construct that will distinguish it from the endogenous gene. This construct was inserted into the vector pBluescript (Stratagene, La Jolla, CA) and transformed into DH10B bacteria. Single colonies were harvested and used to prepare larger quantities of targeting vector.

## 2. Preparation of TCCR -/- stem cells

The targeting vector was linearized by digestion with the restriction endonuclease NotII and transfected into embryonic stem (ES) cells. ES cells are chosen for their ability to integrate into the germ line of developing embryos so as to transmit the targeting vector to their progeny. The preferred ES line of choice is the ESGS line but the D3 line (ATCC CRL-1934) may also be used. Electroporation is done by using 2-5 million ES cells resuspended in 0.8 ml PBS. The linearized targeting vector (20 µg) is added to the cells and this is placed in a sterile electroporation cuvette (0.4 cm Bio-Rad, Hercules, CA). Electroporation is performed using the Bio-Rad electroporation apparatus set at 500 µF, 240 volts. The contents of the cuvette are transferred into 410 ml of ES media. ES media is composed of: High glucose DMEM (Gibco 11960-010), 10% FBS (ES cell tested Gibco 16141-061) and 1000 units/ml ESGRO murine LIF (Gibco 13275-0290). These cells are then aliquoted into 20 96 well dishes. After transfection of the targeting vector the ES cells are selected for by using a lethal concentration of previously mentioned drugs. In the instance of G418, 400 µg/ml is used. Only those ES cells carrying the targeting vector will have the antibiotic resistance markers necessary for survival. The selected ES cell colonies are then screened for correct integration of the vector via southern blotting (Fig. 10A), PCR (Fig. 10B), lack of endogenous target gene mRNA expression (Fig. 10C). ES clones that pass the above criteria are then used for microinjection into embryos.

## 3. Injection and screening of TCCR -/- mice

Selected and screened ES cell colonies from the previous step are transferred into a developing embryo by any suitable technique in art, preferably by microinjection. Suitable microinjection techniques are described in Hogan *et al.*, *Manipulating the mouse embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1986. While any embryo may be used provided that it can be later identified, preferably the embryos selected for microinjection are male and have a coat color that is opposite of the coat color encoded by the genes of the ES cell containing the targeting vector. For example, ES cells from an animal with white fur would be injected into an embryo that will develop brown/black fur. In this manner successfully microinjected embryos can be selected as matured adults on the basis of a mosaic coat color. The resulting mosaic animals (founders) are TCCR +/- and are then backcrossed (mated with other TCCR +/- progeny) to create TCCR -/- mice. To confirm the TCCR -/- genotype, DNA is extracted from tail clippings which is effected by incubating tail tissue at 60°C overnight in 0.5 ml of lysis buffer. The lysis buffer consists of 0.5% SDS, 100 mM NaCl, 50 mM Tris-HCL



(pH 8.0), 7.5 mM EDTA (pH 8.0) and 1 mg/ml proteinase K (Boehringer-Mannheim). After overnight incubation, an aliquots of 75 µl of 8M potassium acetate, 600 ml of CHCl<sub>3</sub> are mixed in the entire reaction is centrifuged for 10 minutes at room temperature. The aqueous layer is removed and placed in a separate eppendorf tube, to which is added 600 ml of 100% ethanol and the DNA is precipitated by centrifugation for 5 minutes. The DNA pellet is washed with 70% ethanol and allowed to air dry. After removal of residual ethanol the DNA pellet is resuspended in 150-200 µl of water. This DNA can then be used for Southern blotting and for PCR analysis. For the Southern blot, the neo gene may be used as a probe; for the PCR, the primers used for screening the ES cells are employed.

The results are reported in Figures 10A, 10B and 10C indicating a successful ablation of the TCCR gene. TCCR-deficient mice were viable, fertile and displayed no overt abnormalities. Detailed histological examination did not reveal any obvious defects. Flow cytometry analysis of cells obtained from thymus, spleen, lymph nodes and peyer's patches of multiple wild-type and knockout mice stained with antibodies to CD3, CD4, CD8, CD25, CD19, B220, CD40, NK1.1, DX5, F4/80, CD14, CD16, MHC II and CD45 did not reveal any gross differences between the two genotypes.

### **EXAMPLE 3**

#### **Enhanced Allergic Airway Inflammation in TCCR <sup>-/-</sup> mice**

Asthma is a complex disease resulting from the interaction of a multitude of allergic and non-allergic factors that elicit bronchial obstruction and inflammation. One of the key aspects of airway inflammation is the infiltration of the airway wall by Th2 cells. Because the TCCR <sup>-/-</sup> mice produce herein have a greater Th2 response, they are a useful model to study allergic airway inflammation.

*Animals:* Twelve TCCR <sup>-/-</sup> mice and eleven wild type littermate (WT) randomly divided into the following four groups: Group 1 - Non-sensitized TCCR <sup>-/-</sup>; Group 2 - Non sensitized TCCR WT (n=4); Group 3 - Sensitized TCCR <sup>-/-</sup> (n=8); and Group 4 - sensitized TCCR WT (n=7).

*Sensitization:* 15 mice (male and female) were sensitized with 300 units/ml of dust mite antigen (Bayer Pharmaceutical) adsorbed to 1 mg/ml Alum given IP at day 0 in 0.1 ml volume. The non sensitized control mice (n=8) received 0.1 ml of 0.9% NaCl and 1 mg/ml Alum IP. Both groups of mice were boosted on day 7 with an IP injection of antigen (sensitized groups) or NaCl (non sensitized groups) as described above.

*Inhalation Challenges:* After sensitization and boost, four DMA inhalation challenges were administered starting on day 16. For aerosolization, the final concentration of dust mite in the nebulizer was 6000 units/ml after being diluted with Dulbecco's PBS and 0.1% of Tween<sup>®</sup>-20. All inhalation challenges were administered in a Plexiglas<sup>®</sup> pie exposure chamber. DMA was aerosolized for 20 minutes using a PARI IS-2 nebulizer initially and then refilled with 1.5 ml, 10 minutes into the exposure. Total deposited dose in the lung was ~ 6.5 AU of DMA.

*AHR (paralyzed):* On day 24, approximately 18 hours after the last DMA aerosol challenge the mice were anesthetized with a mixture of pentobarbital (25 mg/kg) and urethane (1.8 g/kg) and catheterized with a 1 cm incision over the right jugular vein. The jugular vein was dissected free and a catheter (PE-10 connected to PE-50) was inserted and tied into place. Additionally, the mice were tracheotomized (1 cm neck incision, trachea dissected free and a cannula inserted and tied into place). The mice were then loaded into a Plexiglas<sup>®</sup> flow plethysmograph for measurement of thoracic expansion and airway pressure. The mice were ventilated using 100% oxygen at a frequency of 170 bpm and Vt equal to 9 µl/gm. Breathing mechanics (lung resistance and dynamic compliance) were

continuously monitored using a computerized (Buxco Electronics) data acquisition program. After baseline measurements, the mice received a one-time 10-second dose of the methacholine (MCH dose of 500 µg/kg) using 200 µg/ml MCH as the stock concentration.

*Sacrifice:* After completion of the airway reactivity measurement EDTA tubes were used to collect blood via the retro-orbital sinus to obtain serum. The abdomen was opened, the descending aorta severed and the diaphragm cut. After time elapsed for the animals to exsanguinate, bronchioalveolar lavage (BAL) was performed. The lungs were lavaged three times with the same bolus of sterile saline (30 µg/g mouse weight) through the previously inserted tracheal cannula. The bolus filled the lung to approximately 70% total lung capacity. The samples of BAL (return averaged 80%) were centrifuged at 1000 x g and 4 °C for 10 minutes. The supernatants were decanted and immediately frozen at -80 °C. The cell pellets were resuspended in 250 µl of PBS with 2% BSA (Sigman, St. Louis, MO), then enumerated using an automated counter (Baker Instruments, Allentown, PA), and recorded as total number of BAL cells/µl. The cell suspension was then adjusted to 200 cells/µl and 100 µl was centrifuged onto coated Superfrost Plus microscope slides (Baxter Diagnostics, Deerfield, IL) at 800 x g for 10 minutes using a cytospin (Shandon, Inc., Pittsburgh, PA). Slides were air dried, fixed for 1 minute in 100% methanol, and stained with Diff-Quik™ (Baxter Health Care, Miami, FL). At least 200 cells were evaluated per slide to obtain a differential leukocyte count.

After BAL, the right lung, spleen and trachea bronchial lymph nodes were removed and frozen in liquid nitrogen for mRNA analysis (and then placed on dry ice). Tail cuts were taken and frozen on dry ice for later genotyping. The remaining left lungs of the mice were removed to evaluate and compare the severity and character of pathologic changes in lungs between experimental groups. This was accomplished by initial fixing of the lung tissue in 10% neutral-buffered formalin, embedded in paraffin, and 3 µm sections were stained with hematoxylin and eosin. Lung sections were taken along the primary bronchus and the entire section was evaluated blindly and scored based on the severity of the inflammation around the airways and blood vessels. The extent of airway epithelial cell hypertrophy using a scale from 0 (no inflammation and airway changes) to 4 (marked inflammation and airway changes).

*IgE ELISA:* For the total IgE sandwich ELISA, the BAL fluid or serum sample was used either undiluted or diluted 1:2 to 1:20 (BAL) and 1:25 to 1:200 (serum) in ELISA buffer. The capture antibody was rabbit anti-mouse IgE (2 µg/ml PBS) and plates were coated for 24-48 hours at 4 °C. The standard was murine IgE (PharMingen, San Diego, CA) which was diluted serially 1:2, starting with 100 ng/ml concentration. The detection antibody, biotinylated FcεRI-IgG was used at a dilution of 1:2000 for 1-1.5 hours. HRP-SA and enzyme development steps were identical to those used for the cytokine assays.

The results demonstrate a significant increase in lymphocyte infiltration into the lung in the TCCR <sup>-/-</sup> mice than in the wild type (Figure 11).

#### **EXAMPLE 4**

##### **Expression of TCCR in *E. coli***

This example illustrates preparation of an unglycosylated form of TCCR by recombinant expression in *E. coli*. The DNA sequence encoding TCCR is initially amplified using selected PCR primers. The primers should contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector. A variety of expression vectors may be employed. An example of a suitable vector is pBR322 (derived from *E. coli*; see

Bolivar *et al.*, *Gene*, 2:95 (1977)) which contains genes for ampicillin and tetracycline resistance. The vector is digested with restriction enzyme and dephosphorylated. The PCR amplified sequences are then ligated into the vector. The vector will preferably include sequences which encode for an antibiotic resistance gene, a trp promoter, a polyhis leader (including the first six STII codons, polyhis sequence, and enterokinase cleavage site), the TCCR coding region, 5 lambda transcriptional terminator, and an argU gene.

The ligation mixture is then used to transform a selected *E. coli* strain using the methods described in Sambrook *et al.*, *supra*. Transformants are identified by their ability to grow on LB plates and antibiotic resistant colonies are then selected. Plasmid DNA can be isolated and confirmed by restriction analysis and DNA sequencing.

10 Selected clones can be grown overnight in liquid culture medium such as LB broth supplemented with antibiotics. The overnight culture may subsequently be used to inoculate a larger scale culture. The cells are then grown to a desired optical density, during which the expression promoter is turned on.

After culturing the cells for several more hours, the cells can be harvested by centrifugation. The cell pellet obtained by the centrifugation can be solubilized using various agents known in the art, and the solubilized TCCR protein can then be purified using a metal chelating column under conditions that allow tight binding of the protein. 15 TCCR may also be expressed in *E. coli* in a poly-His tagged form, using the following procedure. The DNA encoding TCCR is initially amplified using selected PCR primers. The primers contain restriction enzyme sites which correspond to the restriction enzyme sites on the selected expression vector, and other useful sequences providing for efficient and reliable translation initiation, rapid purification on a metal chelation column, and proteolytic removal with enterokinase. The PCR-amplified, poly-His tagged sequences are then ligated into an expression vector, which 20 is used to transform an *E. coli* host based on strain 52 (W3110 fuhA(tonA) lon galE rpoHts(htpRts) clpP(lacIq). Transformants are first grown in LB containing 50 mg/ml carbenicillin at 30°C with shaking until an O.D.600 of 3-5 is reached. Cultures are then diluted 50-100 fold into CRAP media (prepared by mixing 3.57 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.71 g sodium citrate 2H<sub>2</sub>O, 1.07 g KCl, 5.36 g Difco yeast extract, 5.36 g Sheffield hycase SF in 500 mL water, as well as 110 mM MPOS, pH 7.3, 0.55% (w/v) glucose and 7 mM MgSO<sub>4</sub>) and grown for approximately 20-30 hours at 30°C 25 with shaking. Samples are removed to verify expression by SDS-PAGE analysis, and the bulk culture is centrifuged to pellet the cells. Cell pellets are frozen until purification and refolding.

*E. coli* paste from 0.5 to 1 L fermentations (6-10 g pellets) is resuspended in 10 volumes (w/v) in 7 M guanidine, 20 mM Tris, pH 8 buffer. Solid sodium sulfite and sodium tetrathionate is added to make final concentrations of 0.1M and 0.02 M, respectively, and the solution is stirred overnight at 4°C. This step results in a denatured protein with all 30 cysteine residues blocked by sulfitolization. The solution is centrifuged at 40,000 rpm in a Beckman Ultracentrifuge for 30 min. The supernatant is diluted with 3-5 volumes of metal chelate column buffer (6 M guanidine, 20 mM Tris, pH 7.4) and filtered through 0.22 micron filters to clarify. Depending on condition, the clarified extract is loaded onto a 5 ml Qiagen Ni-NTA metal chelate column equilibrated in the metal chelate column buffer. The column is washed with additional buffer containing 50 mM imidazole (Calbiochem, Utrol grade), pH 7.4. The protein is eluted with 35 buffer containing 250 mM imidazole. Fractions containing the desired protein was pooled and stored at 4°C. Protein concentration is estimated by its absorbance at 280 nm using the calculated extinction coefficient based on its amino acid sequence.

The proteins are refolded by diluting sample slowly into freshly prepared refolding buffer consisting of: 20

mM Tris, pH 8.6, 0.3 M NaCl, 2.5 M urea, 5 mM cysteine, 20 mM glycine and 1 mM EDTA. Refolding volumes are chosen so that the final protein concentration is between 50 to 100 micrograms/ml. The refolding solution is stirred gently at 4 C for 12-36 hours. The refolding reaction is quenched by the addition of TFA to a final concentration of 0.4% (pH of approximately 3). Before further purification of the protein, the solution is filtered through a 0.22 micron filter and acetonitrile is added to 2-10% final concentration. The refolded protein is chromatographed on a Poros R1/H reversed phase column using a mobile buffer of 0.1% TFA with elution with a gradient of acetonitrile from 10 to 80%. Aliquots of fractions with A280 absorbance are analyzed on SDS polyacrylamide gels and fractions containing homogeneous refolded protein are pooled. Generally, the properly refolded species of most proteins are eluted at the lowest concentrations of acetonitrile since those species are the most compact with their hydrophobic interiors shielded from interaction with the reversed phase resin. Aggregated species are usually eluted at higher acetonitrile concentrations. In addition to resolving misfolded forms of proteins from the desired form, the reversed phase step also removes endotoxin from the samples.

Fractions containing the desired folded TCCR proteins, respectively, are pooled and the acetonitrile removed using a gentle stream of nitrogen directed at the solution. Proteins are formulated into 20 mM Hepes, pH 6.8 with 0.14 M sodium chloride and 4% mannitol by dialysis or by gel filtration using G25 Superfine (Pharmacia) resins equilibrated in the formulation buffer and sterile filtered.

#### **EXAMPLE 5**

##### **Expression of TCCR in mammalian cells**

This example illustrates preparation of a potentially glycosylated form of TCCR by recombinant expression in mammalian cells.

The vector, pRK5 (see EP 307,247, published March 15, 1989), is employed as the expression vector. Optionally, the TCCR DNA is ligated into pRK5 with selected restriction enzymes to allow insertion of the TCCR DNA using ligation methods such as described in Sambrook *et al.*, *supra*. The resulting vector is called, for example, pRK5-TCCR.

In one embodiment, the selected host cells may be 293 cells. Human 293 cells (ATCC CCL 1573) are grown to confluence in tissue culture plates in medium such as DMEM supplemented with fetal calf serum and optionally, nutrient components and/or antibiotics. About 10 µg pRK5-TCCR DNA is mixed with about 1 µg DNA encoding the VA RNA gene [Thimmappaya *et al.*, *Cell*, 31:543 (1982)] and dissolved in 500 µL of 1 mM Tris-HCl, 0.1 mM EDTA, 0.227 M CaCl<sub>2</sub>. To this mixture is added, dropwise, 500 µL of 50 mM HEPES (pH 7.35), 280 mM NaCl, 1.5 mM NaPO<sub>4</sub>, and a precipitate is allowed to form for 10 minutes at 25°C. The precipitate is suspended and added to the 293 cells and allowed to settle for about four hours at 37°C. The culture medium is aspirated off and 2 ml of 20% glycerol in PBS is added for 30 seconds. The 293 cells are then washed with serum free medium, fresh medium is added and the cells are incubated for about 5 days.

Approximately 24 hours after the transfections, the culture medium is removed and replaced with culture medium (alone) or culture medium containing 200 µCi/ml <sup>35</sup>S-cysteine and 200 µCi/ml <sup>35</sup>S-methionine. After a 12 hour incubation, the conditioned medium is collected, concentrated on a spin filter, and loaded onto a 15% SDS gel. The processed gel may be dried and exposed to film for a selected period of time to reveal the presence of TCCR polypeptide. The cultures containing transfected cells may undergo further incubation (in serum free medium) and

the medium is tested in selected bioassays.

In an alternative technique, TCCR may be introduced into 293 cells transiently using the dextran sulfate method described by Sompanyrac *et al.*, *Proc. Natl. Acad. Sci.*, 12:7575 (1981). 293 cells are grown to maximal density in a spinner flask and 700 µg pRK5-TCCR DNA is added. The cells are first concentrated from the spinner flask by centrifugation and washed with PBS. The DNA-dextran precipitate is incubated on the cell pellet for four hours. The cells are treated with 20% glycerol for 90 seconds, washed with tissue culture medium, and re-introduced into the spinner flask containing tissue culture medium, 5 µg/ml bovine insulin and 0.1 µg/ml bovine transferrin. After about four days, the conditioned media is centrifuged and filtered to remove cells and debris. The sample containing expressed TCCR can then be concentrated and purified by any selected method, such as dialysis and/or column chromatography.

In another embodiment, TCCR can be expressed in CHO cells. The pRK5-TCCR can be transfected into CHO cells using known reagents such as  $\text{CaPO}_4$  or DEAE-dextran. As described above, the cell cultures can be incubated, and the medium replaced with culture medium (alone) or medium containing a radiolabel such as  $^{35}\text{S}$ -methionine. After determining the presence of TCCR, the culture medium may be replaced with serum free medium. Preferably, the cultures are incubated for about 6 days, and then the conditioned medium is harvested. The medium containing the expressed TCCR can then be concentrated and purified by any selected method.

Epitope-tagged TCCR may also be expressed in host CHO cells. The TCCR may be subcloned out of the pRK5 vector. The subclone insert can undergo PCR to fuse in frame with a selected epitope tag such as a poly-his tag into a Baculovirus expression vector. The poly-his tagged TCCR insert can then be subcloned into a SV40 driven vector containing a selection marker such as DHFR for selection of stable clones. Finally, the CHO cells can be transfected (as described above) with the SV40 driven vector. Labeling may be performed, as described above, to verify expression. The culture medium containing the expressed poly-His tagged TCCR can then be concentrated and purified by any selected method, such as by  $\text{Ni}^{2+}$ -chelate affinity chromatography.

TCCR may also be expressed in CHO and/or COS cells by a transient expression procedure or in CHO cells by another stable expression procedure.

Stable expression in CHO cells may be performed using the procedure outlined below. The proteins may be expressed, for example, either (1) as an IgG construct (immunoadhesion), in which the coding sequences for the soluble forms (*e.g.*, extracellular domains) of the respective proteins are fused to an IgG constant region sequence containing the hinge CH2 domain and/or (2) a poly-His tagged form.

Following PCR amplification, the respective DNAs are subcloned in a CHO expression vector using standard techniques as described in Ausubel *et al.*, *Current Protocols of Molecular Biology*, Unit 3.16, John Wiley and Sons (1997). CHO expression vectors are constructed to have compatible restriction sites 5' and 3' of the DNA of interest to allow the convenient shuttling of cDNAs. The vector used expression in CHO cells is as described in Lucas *et al.*, *Nucl. Acids Res.* 24:9 (1774-1779 (1996), and uses the SV40 early promoter/enhancer to drive expression of the cDNA of interest and dihydrofolate reductase (DHFR). DHFR expression permits selection for stable maintenance of the plasmid following transfection.

Twelve micrograms of the desired plasmid DNA is introduced into approximately 10 million CHO cells using commercially available transfection reagents Superfect® (Quiagen), Dospert® or Fugene® (Boehringer

Mannheim). The cells are grown as described in Lucas *et al.*, *supra*. Approximately  $3 \times 10^7$  cells are frozen in an ampule for further growth and production as described below.

The ampules containing the plasmid DNA are thawed by placement into water bath and mixed by vortexing. The contents are pipetted into a centrifuge tube containing 10 mLs of media and centrifuged at 1000 rpm for 5 minutes. The supernatant is aspirated and the cells are resuspended in 10 mL of selective media (0.2  $\mu$ m filtered PS20 with 5% 0.2  $\mu$ m diafiltered fetal bovine serum). The cells are then aliquoted into a 100 mL spinner containing 90 mL of selective media. After 1-2 days, the cells are transferred into a 250 mL spinner filled with 150 mL selective growth medium and incubated at 37°C. After another 2-3 days, 250 mL, 500 mL and 2000 mL spinners are seeded with  $3 \times 10^5$  cells/mL. The cell media is exchanged with fresh media by centrifugation and resuspension in production medium. Although any suitable CHO media may be employed, a production medium described in U.S. Patent No. 5,122,469, issued June 16, 1992 may actually be used. A 3L production spinner is seeded at  $1.2 \times 10^6$  cells/mL. On day 0, the cell number pH is determined. On day 1, the spinner is sampled and sparging with filtered air is commenced. On day 2, the spinner is sampled, the temperature shifted to 33°C, and 30 mL of 500 g/L glucose and 0.6 mL of 10% antifoam (*e.g.*, 35% polydimethylsiloxane emulsion, Dow Corning 365 Medical Grade Emulsion) taken. Throughout the production, the pH is adjusted as necessary to keep it at around 7.2. After 10 days, or until the viability dropped below 70%, the cell culture is harvested by centrifugation and filtering through a 0.22  $\mu$ m filter. The filtrate was either stored at 4°C or immediately loaded onto columns for purification.

For the poly-His tagged constructs, the proteins are purified using a Ni-NTA column (Qiagen). Before purification, imidazole is added to the conditioned media to a concentration of 5 mM. The conditioned media is pumped onto a 6 ml Ni-NTA column equilibrated in 20 mM Hepes, pH 7.4, buffer containing 0.3 M NaCl and 5 mM imidazole at a flow rate of 4-5 ml/min. at 4°C. After loading, the column is washed with additional equilibration buffer and the protein eluted with equilibration buffer containing 0.25 M imidazole. The highly purified protein is subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8, with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.

Immunoadhesin (Fc-containing) constructs are purified from the conditioned media as follows. The conditioned medium is pumped onto a 5 ml Protein A column (Pharmacia) which had been equilibrated in 20 mM Na phosphate buffer, pH 6.8. After loading, the column is washed extensively with equilibration buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein is immediately neutralized by collecting 1 ml fractions into tubes containing 275  $\mu$ L of 1 M Tris buffer, pH 9. The highly purified protein is subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity is assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.

## EXAMPLE 6

### Expression of TCCR in Yeast

The following method describes recombinant expression of TCCR in yeast.

First, yeast expression vectors are constructed for intracellular production or secretion of TCCR from the ADH2/GAPDH promoter. DNA encoding TCCR and the promoter is inserted into suitable restriction enzyme sites in the selected plasmid to direct intracellular expression of TCCR. For secretion, DNA encoding TCCR can be cloned into the selected plasmid, together with DNA encoding the ADH2/GAPDH promoter, a native TCCR signal peptide

or other mammalian signal peptide, or, for example, a yeast alpha-factor or invertase secretory signal/leader sequence, and linker sequences (if needed) for expression of TCCR.

Yeast cells, such as yeast strain AB110, can then be transformed with the expression plasmids described above and cultured in selected fermentation media. The transformed yeast supernatants can be analyzed by precipitation with 10% trichloroacetic acid and separation by SDS-PAGE, followed by staining of the gels with Coomassie Blue stain.

Recombinant TCCR can subsequently be isolated and purified by removing the yeast cells from the fermentation medium by centrifugation and then concentrating the medium using selected cartridge filters. The concentrate containing TCCR may further be purified using selected column chromatography resins.

#### EXAMPLE 7

##### Expression of TCCR in Baculovirus-Infected Insect Cells

The following method describes recombinant expression of TCCR in Baculovirus-infected insect cells.

The sequence coding for TCCR is fused upstream of an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-his tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pVL1393 (Novagen). Briefly, the sequence encoding TCCR or the desired portion of the coding sequence of TCCR [such as the sequence encoding the extracellular domain of a transmembrane protein or the sequence encoding the mature protein if the protein is extracellular] is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product is then digested with those selected restriction enzymes and subcloned into the expression vector.

Recombinant baculovirus is generated by co-transfecting the above plasmid and BaculoGold™ virus DNA (Pharmingen) into *Spodoptera frugiperda* ("Sf9") cells (ATCC CRL 1711) using lipofectin (commercially available from GIBCO-BRL). After 4 - 5 days of incubation at 28°C, the released viruses are harvested and used for further amplifications. Viral infection and protein expression are performed as described by O'Reilley *et al.*, *Baculovirus expression vectors: A Laboratory Manual*, Oxford: Oxford University Press (1994).

Expressed poly-his tagged TCCR can then be purified, for example, by Ni<sup>2+</sup>-chelate affinity chromatography as follows. Extracts are prepared from recombinant virus-infected Sf9 cells as described by Rupert *et al.*, *Nature*, 362: 175-179 (1993). Briefly, Sf9 cells are washed, resuspended in sonication buffer (25 mL Hepes, pH 7.9; 12.5 mM MgCl<sub>2</sub>; 0.1 mM EDTA; 10% glycerol; 0.1% NP-40; 0.4 M KCl), and sonicated twice for 20 seconds on ice. The sonicates are cleared by centrifugation, and the supernatant is diluted 50-fold in loading buffer (50 mM phosphate, 300 mM NaCl, 10% glycerol, pH 7.8) and filtered through a 0.45 µm filter. A Ni<sup>2+</sup>-NTA agarose column (commercially available from Qiagen) is prepared with a bed volume of 5 mL, washed with 25 mL of water and equilibrated with 25 mL of loading buffer. The filtered cell extract is loaded onto the column at 0.5 mL per minute. The column is washed to baseline A<sub>280</sub> with loading buffer, at which point fraction collection is started. Next, the column is washed with a secondary wash buffer (50 mM phosphate; 300 mM NaCl, 10% glycerol, pH 6.0), which elutes nonspecifically bound protein. After reaching A<sub>280</sub> baseline again, the column is developed with a 0 to 500 mM Imidazole gradient in the secondary wash buffer. One mL fractions are collected and analyzed by SDS-PAGE and

silver staining or Western blot with  $\text{Ni}^{2+}$ -NTA-conjugated to alkaline phosphatase (Qiagen). Fractions containing the eluted His<sub>10</sub>-tagged TCCR are pooled and dialyzed against loading buffer.

Alternatively, purification of the IgG tagged (or Fc tagged) TCCR can be performed using known chromatography techniques, including for instance, Protein A or Protein G column chromatography.

5 Alternatively still, the TCCR molecules of the invention may be expressed using a modified baculovirus procedure employing Hi-5 cells. In this procedure, the DNA encoding the desired sequence was amplified with suitable systems, such as Pfu (Stratagene), or fused upstream (5'-of) an epitope tag contained within a baculovirus expression vector. Such epitope tags include poly-His tags and immunoglobulin tags (like Fc regions of IgG). A variety of plasmids may be employed, including plasmids derived from commercially available plasmids such as pIE-1  
10 (Novagen). The pIE1-1 and pIE1-2 vectors are designed for constitutive expression of recombinant proteins from the baculovirus ie1 promoter in stably transformed insect cells. The plasmids differ only in the orientation of the multiple cloning sites and contain all promoter sequences known to be important for ie1-mediated gene expression in uninfected insect cells as well as the hr5 enhancer element. pIE1-1 and pIE1-2 include the ie1 translation initiation site and can be used to produce fusion proteins. Briefly, the desired sequence or the desired portion of the sequence (such as the  
15 sequence encoding the extracellular domain of the transmembrane protein) is amplified by PCR with primers complementary to the 5' and 3' regions. The 5' primer may incorporate flanking (selected) restriction enzyme sites. The product was then digested with those selected restriction enzymes and subcloned into the expression vector. For example, derivatives of pIE1-1 can include the Fc region of human IgG (pb.PH.IgG) or an 8 histidine (pb.PH.His) tag downstream (3'-of) the desired sequence. Preferably, the vector construct is sequenced for confirmation.

20 Hi5 cells are grown to a confluency of 50% under the conditions of 27 °C, no CO<sub>2</sub>, no pen/strep. For each 150 mm plate, 30 µg of pIE based vector containing the sequence was mixed with 1 ml Ex-Cell medium (Media: Ex-Cell 401 + 1/100 L-Glu JRH Biosciences #14401-78P (note: this media is light sensitive)). Separately, 100 µl of Cell Fectin (CellFECTIN, Gibco BRL +10362-010, pre-vortexed) is mixed with 1 ml of Ex-Cell medium. The two solutions are combined and incubated at room temperature for 15 minutes. 8 ml of Ex-Cell media is added to the 2  
25 ml of DNA/CellFECTIN mix and this is layered on Hi5 cells that have been washed once with Ex-Cell media. The plate is then incubated in darkness for 1 hour at room temperature. The DNA/CellFECTIN mix is then aspirated, and the cells are washed once with Ex-Cell to remove excess Cell FECTIN. 30 ml of fresh Ex-Cell media is added and the cells are incubated for 3 days at 28°C. The supernatant is harvested and the expression of the sequence in the baculovirus expression vector is determined by batch binding of 1 ml of supernatant to 25 ml of Ni-NTA beads (QIAGEN) for histidine tagged proteins of Protein-A Sepharose CL-4B beads (Pharmacia) for IgG tagged proteins followed by SDS-PAGE analysis comparing to a known concentration of protein standard by Coomassie blue staining.

30 The conditioned media from the transfected cells (0.5 to 3 L) was harvested by centrifugation to remove the cells and filtered through 0.22 micron filters. For the poly-His tagged constructs, the protein comprising the sequence is purified using a Ni-NTA column (Qiagen). Before purification, imidazole at a flow rate of 4-5 ml/min. at 48°C. After loading, the column is washed with additional equilibrium buffer and the protein eluted with equilibrium buffer  
35 containing 0.25M imidazole. The highly purified protein was then subsequently desalted into a storage buffer containing 10 mM Hepes, 0.14 M NaCl and 4% mannitol, pH 6.8 with a 25 ml G25 Superfine (Pharmacia) column and stored at -80°C.



Immunoadhesion (Fc-containing) constructs may also be purified from the conditioned media as follows: The conditioned media is pumped onto a 5 ml Protein A column (Pharmacia) which had been previously equilibrated in 20 mM sodium phosphate buffer, pH 6.8. After loading, the column is washed extensively with equilibrium buffer before elution with 100 mM citric acid, pH 3.5. The eluted protein is immediately neutralized by collecting 1 ml fractions into tubes containing 275 µl of 1 M Tris buffer, pH 9. The highly purified protein is subsequently desalted into storage buffer as described above for the poly-His tagged proteins. The homogeneity is assessed by SDS polyacrylamide gels and by N-terminal amino acid sequencing by Edman degradation.

#### **EXAMPLE 8**

##### **Preparation of Antibodies that Bind TCCR**

10 This example illustrates preparation of monoclonal antibodies which can specifically bind TCCR.

Techniques for producing the monoclonal antibodies are known in the art and are described, for instance, in Goding, *supra*. Immunogens that may be employed include purified TCCR, fusion proteins containing TCCR, and cells expressing recombinant TCCR on the cell surface. Selection of the immunogen can be made by the skilled artisan without undue experimentation.

15 Mice, such as Balb/c, are immunized with the TCCR immunogen emulsified in complete Freund's adjuvant and injected subcutaneously or intraperitoneally in an amount from 1-100 micrograms. Alternatively, the immunogen is emulsified in MPL-TDM adjuvant (Ribi Immunochemical Research, Hamilton, MT) and injected into the animal's hind foot pads. The immunized mice are then boosted 10 to 12 days later with additional immunogen emulsified in the selected adjuvant. Thereafter, for several weeks, the mice may also be boosted with additional immunization injections. Serum samples may be periodically obtained from the mice by retro-orbital bleeding for testing in ELISA assays to detect anti-TCCR antibodies.

20 After a suitable antibody titer has been detected, the animals "positive" for antibodies can be injected with a final intravenous injection of TCCR. Three to four days later, the mice are sacrificed and the spleen cells are harvested. The spleen cells are then fused (using 35% polyethylene glycol) to a selected murine myeloma cell line such as P3X63AgU.1, available from ATCC, No. CRL 1597.

25 The fusions generate hybridoma cells which can then be plated in 96 well tissue culture plates containing HAT (hypoxanthine, aminopterin, and thymidine) medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

30 The hybridoma cells are screened in an ELISA for reactivity against TCCR. Determination of "positive" hybridoma cells secreting the desired monoclonal antibodies against TCCR is within the skill in the art.

The positive hybridoma cells can be injected intraperitoneally into syngeneic Balb/c mice to produce ascites containing the anti-TCCR monoclonal antibodies. Alternatively, the hybridoma cells can be grown in tissue culture flasks or roller bottles. Purification of the monoclonal antibodies produced in the ascites can be accomplished using ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can be employed.

**EXAMPLE 9****Purification of TCCR Polypeptides Using Specific Antibodies**

Native or recombinant TCCR polypeptides may be purified by a variety of standard techniques in the art of protein purification. For example, pro-TCCR polypeptide, mature TCCR polypeptide, or pre-TCCR polypeptide can be purified by immunoaffinity chromatography using antibodies specific for the TCCR polypeptide of interest. In general, an immunoaffinity column is constructed by covalently coupling the anti-TCCR polypeptide antibody to an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, N.J.). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated SEPHAROSE™ (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such an immunoaffinity column is utilized in the purification of TCCR polypeptide by preparing a fraction from cells containing TCCR polypeptide in a soluble form. This preparation is derived by solubilization of the whole cell or of a subcellular fraction obtained via differential centrifugation by the addition of detergent or by other methods well known in the art. Alternatively, soluble TCCR polypeptide containing a signal sequence may be secreted in useful quantity into the medium in which the cells are grown.

A soluble TCCR polypeptide-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of TCCR polypeptide (*e.g.*, high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt antibody/TCCR polypeptide binding (*e.g.*, a low pH buffer such as approximately pH 2-3, or a high concentration of a chaotrope such as urea or thiocyanate ion), and TCCR polypeptide is collected.

**Example 10****Drug Screening**

Methods may be employed which are particularly useful for screening compounds by using TCCR polypeptides or binding fragments thereof in any of a variety of drug screening techniques. The TCCR polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the TCCR polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example the formation of complexes between TCCR polypeptide or a fragment thereof and the agent being tested. Alternatively, one can examine the diminution in complex formation between the TCCR polypeptide and its target cell or target receptors caused by the agent being tested.

Thus, the present invention provides methods of screening for drugs or any other agents which can affect a TCCR polypeptide-associated disease or disorder. These methods comprise contacting such an agent with a TCCR polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the TCCR polypeptide or fragment, or (ii) for the presence of a complex between the TCCR polypeptide or fragment and the cell,

by methods well known in the art. In such competitive binding assays, the TCCR polypeptide or fragment is typically labeled. After suitable incubation, free TCCR polypeptide or fragment thereof is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of the particular agent to bind to TCCR polypeptide or to interfere with the TCCR polypeptide/cell complex.

5 Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to a polypeptide and is described in detail in WO 84/03564, published on September 13, 1984. Briefly, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. As applied to a TCCR polypeptide, the peptide test compounds are reacted with TCCR polypeptide and washed. Bound TCCR polypeptide is detected by methods well known in the art. Purified TCCR  
10 polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies can be used to capture the peptide and immobilize it on the solid support.

This invention also contemplated the use of competitive drug screening assays in which neutralizing antibodies capable of binding TCCR binding polypeptide specifically compete with a test compound for binding to TCCR polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any  
15 peptide which shares one or more antigenic determinants with TCCR polypeptide.

#### **EXAMPLE 11**

##### **Rational Drug Design**

The goal of rational drug design is to produce structural analogs of biologically active polypeptide of interest (*i.e.*, a TCCR polypeptide) or of small molecules with which they interact, *e.g.*, agonists, antagonists, or inhibitors. Any  
20 of these examples can be used to fashion drugs which are more active or stable forms of the TCCR polypeptide or which enhance or interfere with the function of the TCCR polypeptide *in vivo* (*c.f.*, Hodgson, *BioTechnology* 9: 19-21 (1991)).

In one approach, the three-dimensional structure of the TCCR polypeptide, or of a TCCR polypeptide-inhibitor complex, is determined by x-ray crystallography, by computer modeling, or most typically, by a combination  
25 of these approaches. Both the shape and charges of the TCCR polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of the TCCR polypeptide may be gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous TCCR polypeptide-like molecules or to identify efficient inhibitors. Useful examples of rational drug design may include molecules which have improved activity or stability as shown  
30 by Braxton and Wells, *Biochemistry* 31: 7796-7801 (1992) or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda *et al.*, *J. Biochem.* 113: 742-746 (1993).

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-  
35 ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides would then act as the pharmacore.

By virtue of the present invention, sufficient amounts of the TCCR polypeptide may be made available to perform such analytical studies as X-ray crystallography. In addition, knowledge of the TCCR polypeptide amino acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

5 Table 2(A-D) show hypothetical exemplifications for using the below described method to determine % amino acid sequence identity (Table 2(A-B)) and % nucleic acid sequence identity (Table 2(C-D)) using the ALIGN-2 sequence comparison computer program, wherein "PRO" represents the amino acid sequence of a hypothetical polypeptide of the invention of interest, "Comparison Protein" represents the amino acid sequence of a polypeptide against which the "PRO" polypeptide of interest is being compared, "PRO-DNA" represents a hypothetical "PRO"-  
10 encoding nucleic acid sequence of interest, "Comparison DNA" represents the nucleotide sequence of a nucleic acid molecule against which the "PRO-DNA" nucleic acid molecule of interest is being compared, "X", "Y" and "Z" each represent different hypothetical amino acid residues and "N", "L" and "V" each represent different hypothetical nucleotides.

### **EXAMPLE 12**

#### **Role of TCCR in Generation of an Immune Response**

*T cell responses:* For anti-KLH responses, mice were immunized with 100 µg KLH in saline, in a 1:1 emulsion with CFA, containing 1 mg/ml *Mycobacterium tuberculosis* strain H37Ra, (Difco Laboratories, Detroit, MI) in the hind footpads. After 9 days, the popliteal lymph nodes were removed and cell suspensions were prepared. The lymph node cells were cultured ( $5 \times 10^5$  per well) in various concentration of KLH in DMEM medium supplemented with 5% FCS. Proliferation was measured by addition of 1 µCi of [ $^3$ H]-thymidine (ICN, Irvine, CA) for the last 18  
20 h of a 5-day culture, and incorporation of radioactivity was assayed by liquid scintillation counting. Assays for cytokine production by T cells were conducted by culturing  $5 \times 10^5$  draining lymph node cells either from KLH-primed wild type or TCCR-deficient mice in the presence of indicated amounts of the KLH in 96 well plates in final volume of 200 µl. After 96 hr of culture, 150 µl of culture supernatant was removed from each well and  
25 cytokine levels were determined by ELISA using antibodies from Pharmingen (San Diego, CA), in the recommended conditions.

*In vitro induction of T cell differentiation:* CD4<sup>+</sup> T cells from spleen and lymph nodes from wild type or TCCR-deficient littermates were purified with anti-CD4 magnetic beads (MACS). Purified T cells ( $10^6$  cells/ml) were activated in the presence of irradiated (3000 rad) syngeneic wild-type or knockout APC ( $10^6$ /ml) and ConA (2.5 µg/ml, Boehringer, Mannheim, Germany), or by plating on plates coated with 5 µg/ml anti-CD3 and 1 µg/ml anti-CD28 antibodies. The culture medium was supplemented with IL-2 (20U/ml), IL-12 (3.5ng/ml, R&D Systems) and 500 ng/ml anti-IL-4 antibody (Pharmingen) for Th1 differentiation, and with IL-2 (20U/ml), IL-4 ( $10^3$  U/ml, R&D Systems) and 500 ng/ml of anti-IFN γ antibody (Pharmingen) for Th2 differentiation. After three days, cells were either  
30 lysed for RNA extraction, or were extensively washed, counted, and restimulated at  $10^6$  cells/ml, either in the presence of ConA (2.5 µg/ml) or on plates coated with 5 µg/ml anti-CD3 antibody. After 24 hours, supernatants were harvested and analyzed for the presence of cytokines.

*Total and OVA-specific immunoglobulin levels:* Unimmunized mice at 12 weeks of age or older were bled and serum was analyzed for the presence of various isotypes of immunoglobulins by ELISA. For anti-OVA specific

antibodies, 6 wk old wild type or TCCR-deficient mice were immunized with 100 µg of OVA in complete Freund's adjuvant (i.p.) and 21 day later challenged with 100 µg of OVA in incomplete Freund's adjuvant (i.p.). Seven days after challenge mice were bled and serum was analyzed for presence of OVA-specific antibodies.

*Real time PCR analysis:* Murine splenocytes were separated into T helper cells (CD4 positive, F4/80 negative, 97% pure), B cells (CD19 positive, 97% pure), natural killer cells (NK1.1 positive, 99% pure), and macrophages (F4/80 positive, >95% pure) by FACS, and into cytotoxic T cells (CD8 positive, 85% pure) by MACS. Total RNA was extracted with Qiagen RNeasy columns and digested with DNase I to remove contaminating DNA. RNA was probed for TCCR using Taqman18. All reactions were made in duplicates and normalized to rpl19, a ribosomal housekeeping gene. A no RT control reaction was included and showed that all samples were free of contaminating DNA. The sequence of all primers and probes is described in Figure 19.

Wild type and TCCR-deficient mice were immunized with keyhole limpet hemocyanin (KLH), and draining lymph nodes harvested 9 days later were assessed for cytokine production after *in vitro* stimulation *in vitro* with KLH (Fig. 16A and B). The ability of TCCR-deficient cells to produce IFN was significantly impaired when challenged with KLH, while the production of IL-4 was markedly enhanced. Production of IL-5 and antigen induced proliferation of TCCR-deficient *in vivo* primed lymph node cells were normal (Fig 16C and D). Normal levels of IFN production were measured upon LPS stimulation of TCCR-deficient mice indicating that there seemed to be no intrinsic defects in IFN production in these mice. These results indicate that TCCR-deficient mice are impaired in their ability to mount a Th1 response. The loss of Th1 response is accompanied by an enhanced Th2 response similar to what has been observed in mice deficient in Th1 cytokines such as IL-12 (Magram, J., *et al.*, 1996, *Immunity*, 4:471-81; Wu, C., *et al.*, 1997, *J Immunol.*, 159:1658-65).

In addition to its role in regulating the cellular immune response, IFN is also involved in immunoglobulin (Ig) isotype regulation. In particular, IFN is known to enhance the production of IgG2a antibodies and, to a lesser extent, of IgG3 antibodies (Snapper, C. M., & Paul, W. E., 1987, *Science*, 236:944-7; Huang, S., *et al.*, 1993, *Science*, 259:1742-5). Consistent with a diminished production of IFN by Th1 cells, TCCR-deficient mice had decreased total serum IgG2a concentrations while the levels of all other immunoglobulin isotypes were normal (Fig. 17A). Furthermore, upon *in vivo* challenge with ovalbumin (OVA), TCCR-deficient mice had severely reduced titers of OVA-specific IgG2a (~20% of controls; Fig. 17B).

Th1 response is crucial in the defense against intracellular pathogens such as *Listeria monocytogenes* (*L. monocytogenes*). To further establish the *in vivo* role of TCCR in the control of Th1 response, TCCR-deficient mice and control littermates were infected with a sublethal dose of *L. monocytogenes* ( $3 \times 10^4$  colony forming units (CFU)). Bacterial titers were determined 3 days or nine days after infection and found to be up to  $10^6$ -fold higher in the livers of TCCR-deficient mice (Fig. 17C).

The role of TCCR in mediating the differentiation of a Th1 response *in vitro* was next investigated. CD4+ T cells from wild type and TCCR-deficient mice were differentiated *in vitro* in the presence of irradiated APC under conditions that favor either Th1 or Th2 cell development. After 3-4 days in culture, cells were washed and restimulated with ConA, and 24h later, supernatants were analyzed for the presence of cytokines. When differentiated into Th1 cells, TCCR-deficient lymphocytes produced 80% less IFN- than their wild type littermates (Fig. 18A). In contrast, TCCR-deficient lymphocytes grown in the presence of IL-4 and anti-IFN- antibodies

produced slightly more IL-4. Similar results were obtained with CD4<sup>+</sup> CD45Rb<sup>high</sup> naïve T cells. This effect is intrinsic to the T cells for 2 reasons: First, similar results were obtained when T cells were differentiated in the presence of APC derived from wild type or TCCR-deficient mice. Second, the effect was reproducible in an APC free system where T cell differentiation was carried out using plate-immobilized anti-CD3/CD28 (Fig. 18B). The reduction in IFN production also correlates with a decrease in the number of IFN producing cells as measured by intracellular FACS staining. The observed Th1 deficiency did not appear to be the result of a defect in the IL-12 receptor as both subunits of the receptor were expressed normally in activated T-cells. Since IL-12 could still promote the proliferation of ConA stimulated T cells from wild type and TCCR-deficient mice, there seems to be no defect in the IL-12 signaling pathway in these mice (Fig. 18C and D).

Table 3(A-Q) provides the complete source code for the ALIGN-2 sequence comparison computer program. This source code may be routinely compiled for use on a UNIX operating system to provide the ALIGN-2 sequence comparison computer program.

**Table 2A**

5 PRO XXXXXXXXXXXXXXXX (Length = 15 amino acids)  
 Comparison Protein XXXXXYYYYYYY (Length = 12 amino acids)

% amino acid sequence identity =

10 (the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 15 = 33.3%

**Table 2B**

15 PRO XXXXXXXXXXXX (Length = 10 amino acids)  
 Comparison Protein XXXXXYYYYYYZZYZ (Length = 15 amino acids)

% amino acid sequence identity =

20 (the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 10 = 50%

25

**Table 2C**

30 PRO-DNA NNNNNNNNNNNNNN (Length = 14 nucleotides)  
 Comparison DNA NNNNNLLLLLLLLLL (Length = 16 nucleotides)

% nucleic acid sequence identity =

35 (the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

6 divided by 14 = 42.9%

**Table 2D**

40 PRO-DNA NNNNNNNNNNNN (Length = 12 nucleotides)  
 Comparison DNA NNNNLLLVV (Length = 9 nucleotides)

% nucleic acid sequence identity =

45 (the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the PRO-DNA nucleic acid sequence) =

4 divided by 12 = 33.3%

**Table 3A**

```

/*
 *
 * C-C increased from 12 to 15
 * Z is average of EQ
 * B is average of ND
 * match with stop is _M; stop-stop = 0; J (joker) match = 0
 */
#define _M -8 /* value of a match with a stop */

int _day[26][26] = {
/* A B C D E F G H I J K L M N O P Q R S T U V W X Y Z */
/* A */ { 2, 0, -2, 0, 0, -4, 1, -1, -1, 0, -1, -2, -1, 0, _M, 1, 0, -2, 1, 1, 0, 0, -6, 0, -3, 0},
/* B */ { 0, 3, -4, 3, 2, -5, 0, 1, -2, 0, 0, -3, -2, 2, _M, -1, 1, 0, 0, 0, 0, -2, -5, 0, -3, 1},
/* C */ { -2, -4, 15, -5, -5, -4, -3, -3, -2, 0, -5, -6, -5, -4, _M, -3, -5, -4, 0, -2, 0, -2, -8, 0, 0, -5},
/* D */ { 0, 3, -5, 4, 3, -6, 1, 1, -2, 0, 0, -4, -3, 2, _M, -1, 2, -1, 0, 0, 0, -2, -7, 0, -4, 2},
/* E */ { 0, 2, -5, 3, 4, -5, 0, 1, -2, 0, 0, -3, -2, 1, _M, -1, 2, -1, 0, 0, 0, -2, -7, 0, -4, 3},
/* F */ { -4, -5, -4, -6, -5, 9, -5, -2, 1, 0, -5, 2, 0, -4, _M, -5, -5, -4, -3, -3, 0, -1, 0, 0, 7, -5},
/* G */ { 1, 0, -3, 1, 0, -5, 5, -2, -3, 0, -2, -4, -3, 0, _M, -1, -1, -3, 1, 0, 0, -1, -7, 0, -5, 0},
/* H */ { -1, 1, -3, 1, 1, -2, -2, 6, -2, 0, 0, -2, -2, 2, _M, 0, 3, 2, -1, -1, 0, -2, -3, 0, 0, 2},
/* I */ { -1, -2, -2, -2, -2, 1, -3, -2, 5, 0, -2, 2, 2, -2, _M, -2, -2, -2, -1, 0, 0, 4, -5, 0, -1, -2},
/* J */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* K */ { -1, 0, -5, 0, 0, -5, -2, 0, -2, 0, 5, -3, 0, 1, _M, -1, 1, 3, 0, 0, 0, -2, -3, 0, -4, 0},
/* L */ { -2, -3, -6, -4, -3, 2, -4, -2, 2, 0, -3, 6, 4, -3, _M, -3, -2, -3, -3, -1, 0, 2, -2, 0, -1, -2},
/* M */ { -1, -2, -5, -3, -2, 0, -3, -2, 2, 0, 0, 4, 6, -2, _M, -2, -1, 0, -2, -1, 0, 2, -4, 0, -2, -1},
/* N */ { 0, 2, -4, 2, 1, -4, 0, 2, -2, 0, 1, -3, -2, 2, _M, -1, 1, 0, 1, 0, 0, -2, -4, 0, -2, 1},
/* O */ { _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, 0, _M, _M, _M, _M, _M, _M, _M, _M, _M},
/* P */ { 1, -1, -3, -1, -1, -5, -1, 0, -2, 0, -1, -3, -2, -1, _M, 6, 0, 0, 1, 0, 0, -1, -6, 0, -5, 0},
/* Q */ { 0, 1, -5, 2, 2, -5, -1, 3, -2, 0, 1, -2, -1, 1, _M, 0, 4, 1, -1, -1, 0, -2, -5, 0, -4, 3},
/* R */ { -2, 0, -4, -1, -1, -4, -3, 2, -2, 0, 3, -3, 0, 0, _M, 0, 1, 6, 0, -1, 0, -2, 2, 0, -4, 0},
/* S */ { 1, 0, 0, 0, 0, -3, 1, -1, -1, 0, 0, -3, -2, 1, _M, 1, -1, 0, 2, 1, 0, -1, -2, 0, -3, 0},
/* T */ { 1, 0, -2, 0, 0, -3, 0, -1, 0, 0, 0, -1, -1, 0, _M, 0, -1, -1, 1, 3, 0, 0, -5, 0, -3, 0},
/* U */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* V */ { 0, -2, -2, -2, -2, -1, -1, -2, 4, 0, -2, 2, 2, -2, _M, -1, -2, -2, -1, 0, 0, 4, -6, 0, -2, -2},
/* W */ { -6, -5, -8, -7, -7, 0, -7, -3, -5, 0, -3, -2, -4, -4, _M, -6, -5, 2, -2, -5, 0, -6, 17, 0, 0, -6},
/* X */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, _M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* Y */ { -3, -3, 0, -4, -4, 7, -5, 0, -1, 0, -4, -1, -2, -2, _M, -5, -4, -4, -3, -3, 0, -2, 0, 0, 10, -4},
/* Z */ { 0, 1, -5, 2, 3, -5, 0, 2, -2, 0, 0, -2, -1, 1, _M, 0, 3, 0, 0, 0, 0, -2, -6, 0, -4, 4}
};

```



**Table 3B**

```

/*
*/
#include <stdio.h>
#include <ctype.h>

#define MAXJMP      16      /* max jumps in a diag */
#define MAXGAP      24      /* don't continue to penalize gaps larger than this */
#define JMPS        1024    /* max jmps in an path */
#define MX          4       /* save if there's at least MX-1 bases since last jmp */

#define DMAT        3       /* value of matching bases */
#define DMIS        0       /* penalty for mismatched bases */
#define DINS0       8       /* penalty for a gap */
#define DINS1       1       /* penalty per base */
#define PINS0       8       /* penalty for a gap */
#define PINS1       4       /* penalty per residue */

struct jmp {
    short          n[MAXJMP];    /* size of jmp (neg for del) */
    unsigned short x[MAXJMP];    /* base no. of jmp in seq x */
};
/* limits seq to 2^16 -1 */

struct diag {
    int            score;        /* score at last jmp */
    long           offset;       /* offset of prev block */
    short          ijmp;         /* current jmp index */
    struct jmp     jp;           /* list of jmps */
};

struct path {
    int            spc;          /* number of leading spaces */
    short          n[JMPS];      /* size of jmp (gap) */
    int            x[JMPS];      /* loc of jmp (last elem before gap) */
};

char              *ofile;       /* output file name */
char              *namex[2];    /* seq names: getseqs() */
char              *prog;        /* prog name for err msgs */
char              *seqx[2];     /* seqs: getseqs() */
int               dmax;         /* best diag: nw() */
int               dmax0;        /* final diag */
int               dna;          /* set if dna: main() */
int               cndgaps;      /* set if penalizing cnd gaps */
int               gapx, gapy;    /* total gaps in seqs */
int               len0, len1;    /* seq lens */
int               ngapx, ngapy;  /* total size of gaps */
int               smax;         /* max score: nw() */
int               *xbm;         /* bitmap for matching */
long              offset;       /* current offset in jmp file */
struct            diag          *dx;    /* holds diagonals */
struct            path          pp[2];  /* holds path for seqs */
char              *calloc(), *malloc(), *index(), *strcpy();
char              *getseq(), *g_calloc();

```

**Table 3C**

```

/* Needleman-Wunsch alignment program
*
* usage: progs file1 file2
* where file1 and file2 are two dna or two protein sequences.
* The sequences can be in upper- or lower-case and may contain ambiguity
* Any lines beginning with ';', '>' or '<' are ignored
* Max file length is 65535 (limited by unsigned short x in the jmp struct)
* A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
* Output is in the file "align.out"
*
* The program may create a tmp file in /tmp to hold info about traceback.
* Original version developed under BSD 4.3 on a vax 8650
*/
#include "nw.h"
#include "day.h"

static _dbval[26] = {
    1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

static _pbval[26] = {
    1, 2|(1<<('D'-'A'))|(1<<('N'-'A')), 4, 8, 16, 32, 64,
    128, 256, 0xFFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
    1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
    1<<23, 1<<24, 1<<25|(1<<('E'-'A'))|(1<<('Q'-'A'))
};

main(ac, av)
    int ac;
    char *av[];
{
    prog = av[0];
    if (ac != 3) {
        fprintf(stderr, "usage: %s file1 file2\n", prog);
        fprintf(stderr, "where file1 and file2 are two dna or two protein sequences.\n");
        fprintf(stderr, "The sequences can be in upper- or lower-case\n");
        fprintf(stderr, "Any lines beginning with ';', '>' or '<' are ignored\n");
        fprintf(stderr, "Output is in the file \"align.out\"\n");
        exit(1);
    }
    namex[0] = av[1];
    namex[1] = av[2];
    seqx[0] = getseq(namex[0], &len0);
    seqx[1] = getseq(namex[1], &len1);
    xbm = (dna)? _dbval : _pbval;
    endgaps = 0; /* 1 to penalize endgaps */
    ofile = "align.out"; /* output file */
    nw(); /* fill in the matrix, get the possible jmps */
    readjmps(); /* get the actual jmps */
    print(); /* print stats, alignment */
    cleanup(0); /* unlink any tmp files */
}

```

main

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**Table 3D**

```

/* do the alignment, return best score: main()
* dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
* pro: PAM 250 values
* When scores are equal, we prefer mismatches to any gap, prefer
* a new gap to extending an ongoing gap, and prefer a gap in seqx
* to a gap in seq y.
*/
nw()
{
    char      *px, *py;          /* seqs and ptrs */
    int       *ndely, *dely;     /* keep track of dely */
    int       ndelx, delx;       /* keep track of delx */
    int       *tmp;              /* for swapping row0, row1 */
    int       mis;               /* score for each type */
    int       ins0, ins1;        /* insertion penalties */
    register  id;                /* diagonal index */
    register  ij;                /* jmp index */
    register  *col0, *col1;      /* score for curr, last row */
    register  xx, yy;            /* index into seqs */
    dx = (struct diag *)g_calloc("to get diag", len0+len1+1, sizeof(struct diag));
    ndely = (int *)g_calloc("to get ndely", len1+1, sizeof(int));
    dely = (int *)g_calloc("to get dely", len1+1, sizeof(int));
    col0 = (int *)g_calloc("to get col0", len1+1, sizeof(int));
    col1 = (int *)g_calloc("to get col1", len1+1, sizeof(int));
    ins0 = (dna)? DINS0 : PINS0;
    ins1 = (dna)? DINS1 : PINS1;
    smax = -10000;
    if (endgaps) {
        for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
            col0[yy] = dely[yy] = col0[yy-1] - ins1;
            ndely[yy] = yy;
        }
        col0[0] = 0;          /* Waterman Bull Math Biol 84 */
    }
    else
        for (yy = 1; yy <= len1; yy++)
            dely[yy] = -ins0;
    /* fill in match matrix
    */
    for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
        /* initialize first entry in col
        */
        if (endgaps) {
            if (xx == 1)
                col1[0] = delx = -(ins0+ins1);
            else
                col1[0] = delx = col0[0] - ins1;
            ndelx = xx;
        }
        else {
            col1[0] = 0;
            delx = -ins0;
            ndelx = 0;
        }
    }
}

```

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**Table 3E**

...nw

```

for (py = seqx[1], yy = 1; yy <= len1; py++, yy++) {
    mis = col0[yy-1];
    if (dna)
        mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
    else
        mis += _day[*px-'A'][*py-'A'];

    /* update penalty for del in x seq;
     * favor new del over ongoing del
     * ignore MAXGAP if weighting endgaps
     */
    if (endgaps || ndely[yy] < MAXGAP) {
        if (col0[yy] - ins0 >= dely[yy]) {
            dely[yy] = col0[yy] - (ins0+ins1);
            ndely[yy] = 1;
        } else {
            dely[yy] -= ins1;
            ndely[yy]++;
        }
    } else {
        if (col0[yy] - (ins0+ins1) >= dely[yy]) {
            dely[yy] = col0[yy] - (ins0+ins1);
            ndely[yy] = 1;
        } else
            ndely[yy]++;
    }

    /* update penalty for del in y seq;
     * favor new del over ongoing del
     */
    if (endgaps || ndelx < MAXGAP) {
        if (col1[yy-1] - ins0 >= delx) {
            delx = col1[yy-1] - (ins0+ins1);
            ndelx = 1;
        } else {
            delx -= ins1;
            ndelx++;
        }
    } else {
        if (col1[yy-1] - (ins0+ins1) >= delx) {
            delx = col1[yy-1] - (ins0+ins1);
            ndelx = 1;
        } else
            ndelx++;
    }

    /* pick the maximum score; we're favoring
     * mis over any dcl and delx over dely
     */

```

**Table 3F**

...nw

```

id = xx - yy + len1 - 1;
if (mis >= delx && mis >= dely[yy])
    col1[yy] = mis;
else if (delx >= dely[yy]) {
    col1[yy] = delx;
    ij = dx[id].ijmp;
    if (dx[id].jp.n[0] && (!dna || (ndelx >= MAXJMP
    && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
        dx[id].ijmp++;
        if (++ij >= MAXJMP) {
            writeimps(id);
            ij = dx[id].ijmp = 0;
            dx[id].offset = offset;
            offset += sizeof(struct jmp) + sizeof(offset);
        }
        dx[id].jp.n[ij] = ndelx;
        dx[id].jp.x[ij] = xx;
        dx[id].score = delx;
    }
    else {
        col1[yy] = dely[yy];
        ij = dx[id].ijmp;
        if (dx[id].jp.n[0] && (!dna || (ndely[yy] >= MAXJMP
        && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
            dx[id].ijmp++;
            if (++ij >= MAXJMP) {
                writeimps(id);
                ij = dx[id].ijmp = 0;
                dx[id].offset = offset;
                offset += sizeof(struct jmp) + sizeof(offset);
            }
            dx[id].jp.n[ij] = -ndely[yy];
            dx[id].jp.x[ij] = xx;
            dx[id].score = dely[yy];
        }
        if (xx == len0 && yy < len1) {
            /* last col
            */
            if (endgaps)
                col1[yy] -= ins0+ins1*(len1-yy);
            if (col1[yy] > smax) {
                smax = col1[yy];
                dmax = id;
            }
        }
    }
    if (endgaps && xx < len0)
        col1[yy-1] -= ins0+ins1*(len0-xx);
    if (col1[yy-1] > smax) {
        smax = col1[yy-1];
        dmax = id;
    }
    tmp = col0; col0 = col1; col1 = tmp;
}
(void) free((char *)ndely);
(void) free((char *)dely);
(void) free((char *)col0);
(void) free((char *)col1);
}

```

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**Table 3G**

```

/*
 *
 * print() -- only routine visible outside this module
 *
 * static:
 * getmat() -- trace back best path, count matches: print()
 * pr_align() -- print alignment of described in array p[]: print()
 * dumpblock() -- dump a block of lines with numbers, stars: pr_align()
 * nums() -- put out a number line: dumpblock()
 * putline() -- put out a line (name, [num], seq, [num]): dumpblock()
 * stars() -- put a line of stars: dumpblock()
 * stripname() -- strip any path and prefix from a seqname
 */
#include "nw.h"
#define SPC 3
#define P_LINE 256 /* maximum output line */
#define P_SPC 3 /* space between name or num and seq */
extern _day[26][26];
int olen; /* set output line length */
FILE *fx; /* output file */
print()
{
    int lx, ly, firstgap, lastgap; /* overlap */

    if ((fx = fopen(ofile, "w")) == 0) {
        fprintf(stderr, "%s: can't write %s\n", prog, ofile);
        cleanup(1);
    }
    fprintf(fx, "<first sequence: %s (length = %d)\n", namex[0], len0);
    fprintf(fx, "<second sequence: %s (length = %d)\n", namex[1], len1);
    olen = 60;
    lx = len0;
    ly = len1;
    firstgap = lastgap = 0;
    if (dmax < len1 - 1) { /* leading gap in x */
        pp[0].spc = firstgap = len1 - dmax - 1;
        ly -= pp[0].spc;
    }
    else if (dmax > len1 - 1) { /* leading gap in y */
        pp[1].spc = firstgap = dmax - (len1 - 1);
        lx -= pp[1].spc;
    }
    if (dmax0 < len0 - 1) { /* trailing gap in x */
        lastgap = len0 - dmax0 - 1;
        lx -= lastgap;
    }
    else if (dmax0 > len0 - 1) { /* trailing gap in y */
        lastgap = dmax0 - (len0 - 1);
        ly -= lastgap;
    }
    getmat(lx, ly, firstgap, lastgap);
    pr_align();
}

```

print

**Table 3H**

```

/*
 * trace back the best path, count matches
 */
static
getmat(lx, ly, firstgap, lastgap)                                getmat
    int      lx, ly;                                           /* "core" (minus endgaps) */
    int      firstgap, lastgap;                                /* leading trailing overlap */
{
    int      nm, i0, i1, siz0, siz1;
    char      outx[32];
    double    pct;
    register  n0, n1;
    register char *p0, *p1;
    /* get total matches, score
     */
    i0 = i1 = siz0 = siz1 = 0;
    p0 = seqx[0] + pp[1].spc;
    p1 = seqx[1] + pp[0].spc;
    n0 = pp[1].spc + 1;
    n1 = pp[0].spc + 1;
    nm = 0;
    while ( *p0 && *p1 ) {
        if (siz0) {
            p1++;
            n1++;
            siz0--;    }
        else if (siz1) {
            p0++;
            n0++;
            siz1--;    }
        else {
            if (xbm[*p0-'A']&xbm[*p1-'A'])
                nm++;
            if (n0++ == pp[0].x[i0])
                siz0 = pp[0].n[i0++];
            if (n1++ == pp[1].x[i1])
                siz1 = pp[1].n[i1++];
            p0++;
            p1++;    }
    }
    /* pct homology:
     * if penalizing endgaps, base is the shorter seq
     * else, knock off overhangs and take shorter core
     */
    if (endgaps)
        lx = (len0 < len1)? len0 : len1;
    else
        lx = (lx < ly)? lx : ly;
    pct = 100.*((double)nm)/((double)lx);
    fprintf(fx, "\n");
    fprintf(fx, "<%d match%s in an overlap of %d: %.2f percent similarity\n",
        nm, (nm == 1)? "" : "es", lx, pct);

```

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**Table 3I**

```

fprintf(fx, "<gaps in first sequence: %d", gapx);
if (gapx) {
    (void) sprintf(outh, " (%d %s%s)",
        gapx, (dna)? "base":"residue", (gapx == 1)? "" : "s");
    fprintf(fx, "%s", outh);
}
fprintf(fx, ", gaps in second sequence: %d", gapy);
if (gapy) { (void) sprintf(outh, " (%d %s%s)",
    gapy, (dna)? "base":"residue", (gapy == 1)? "" : "s");
    fprintf(fx, "%s", outh); }

if (dna)
    fprintf(fx,
        "\n<score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
        smax, DMAT, DMIS, DINS0, DINS1);
else
    fprintf(fx,
        "\n<score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
        smax, PINS0, PINS1);

if (endgaps)
    fprintf(fx,
        "<endgaps penalized. left endgap: %d %s%s, right endgap: %d %s%s\n",
        firstgap, (dna)? "base" : "residue", (firstgap == 1)? "" : "s",
        lastgap, (dna)? "base" : "residue", (lastgap == 1)? "" : "s");
else
    fprintf(fx, "<endgaps not penalized\n"); }

static nm; /* matches in core -- for checking */
static lmax; /* lengths of stripped file names */
static ij[2]; /* jmp index for a path */
static nc[2]; /* number at start of current line */
static ni[2]; /* current clem number -- for gapping */
static siz[2];
static char *ps[2]; /* ptr to current element */
static char *po[2]; /* ptr to next output char slot */
static char out[2][P_LINE]; /* output line */
static char star[P_LINE]; /* set by stars() */

/*
 * print alignment of described in struct path pp[ ]
 */
static
pr_align()
{
    int nn; /* char count */
    int more;
    register i;
    for (i = 0, lmax = 0; i < 2; i++) { nn = stripname(name[i]);
        if (nn > lmax)
            lmax = nn;
        nc[i] = 1;
        ni[i] = 1;
        siz[i] = ij[i] = 0;
        ps[i] = seqx[i];
        po[i] = out[i];
    }
}

```

...getmat

pr\_align



**Table 3J**

```

for (nn = nm = 0, more = 1; more; ) {
    for (i = more = 0; i < 2; i++) {
        /*
         * do we have more of this sequence?
         */
        if (!*ps[i])
            continue;
        more++;
        if (pp[i].spe) { /* leading space */
            *po[i]++ = ' ';
            pp[i].spe--;
        }
        else if (siz[i]) { /* in a gap */
            *po[i]++ = '-';
            siz[i]--;
        }
        else { /* we're putting a seq element
            */
            *po[i] = *ps[i];
            if (islower(*ps[i]))
                *ps[i] = toupper(*ps[i]);
            po[i]++;
            ps[i]++;
            /*
             * are we at next gap for this seq?
             */
            if (ni[i] == pp[i].x[ij[i]]) {
                /*
                 * we need to merge all gaps
                 * at this location
                 */
                siz[i] = pp[i].n[ij[i]++];
                while (ni[i] == pp[i].x[ij[i]])
                    siz[i] += pp[i].n[ij[i]++];
            }
            ni[i]++;
        }
    }
    if (++nn == olen || !more && nn) {
        dumpblock();
        for (i = 0; i < 2; i++)
            po[i] = out[i];
        nn = 0;
    }
}
/*
 * dump a block of lines, including numbers, stars: pr_align()
 */
static
dumpblock()
{
    register i;
    for (i = 0; i < 2; i++)
        *po[i]-- = '\0';
}

```

...pr\_align

dumpblock

**Table 3K**

...dumpblock

```

(void) putc('\n', fx);
for (i = 0; i < 2; i++) {
    if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
        if (i == 0)
            nums(i);
        if (i == 0 && *out[1])
            stars();
        putline(i);
        if (i == 0 && *out[1])
            fprintf(fx, star);
        if (i == 1)
            nums(i);
    }
}
}
/*
 * put out a number line: dumpblock()
 */
static
nums(ix)
{
    int      ix;      /* index in out[] holding seq line */
    char      nline[P_LINE];
    register  i, j;
    register char *pn, *px, *py;
    for (pn = nline, i = 0; i < lmax+P_SPC; i++, pn++)
        *pn = ' ';
    for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
        if (*py == ' ' || *py == '\n')
            *pn = ' ';
        else {
            if (i%10 == 0 || (i == 1 && nc[ix] != 1)) {
                j = (i < 0)? -i : i;
                for (px = pn; j; j /= 10, px--)
                    *px = j%10 + '0';
                if (i < 0)
                    *px = '-';
            }
            else
                *pn = ' ';
            i++;
        }
    }
    *pn = '\0';
    nc[ix] = i;
    for (pn = nline; *pn; pn++)
        (void) putc(*pn, fx);
    (void) putc('\n', fx);}
/*
 * put out a line (name, [num], seq, [num]): dumpblock()
 */
static
putline(ix)
{
    int      ix;

```

nums

putline

**Table 3L**

...putline

```

    int          i;
    register char *px;
    for (px = namex[ix], i = 0; *px && *px != '\0'; px++, i++)
        (void) putc(*px, fx);
    for (; i < lmax+P_SPC; i++)
        (void) putc(' ', fx);
    /* these count from 1:
    * ni[] is current element (from 1)
    * nc[] is number at start of current line
    */
    for (px = out[ix]; *px; px++)
        (void) putc(*px&0x7F, fx);
    (void) putc('\n', fx);
}

/*
 * put a line of stars (seqs always in out[0], out[1]): dumpblock()
 */
static
stars()
{
    int          i;
    register char *p0, *p1, cx, *px;

    if (!*out[0] || (*out[0] == ' ' && *(po[0]) == ' ') ||
        !*out[1] || (*out[1] == ' ' && *(po[1]) == ' '))
        return;
    px = star;
    for (i = lmax+P_SPC; i; i--)
        *px++ = ' ';

    for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
        if (isalpha(*p0) && isalpha(*p1)) {
            if (xbm[*p0-'A']&xbm[*p1-'A']) {
                cx = '*';
                nm++;
            }
            else if (!dna && _day[*p0-'A'][*p1-'A'] > 0)
                cx = '.';
            else
                cx = ' ';
        }
        else
            cx = ' ';
        *px++ = cx;
    }
    *px++ = '\n';
    *px = '\0';
}

```

stars

**Table 3M**

```

/*
 * strip path or prefix from pn, return len: pr_align()
 */
static
stripname(pn)                                stripname
    char    *pn;    /* file name (may be path) */
{
    register char    *px, *py;

    py = 0;
    for (px = pn; *px; px++)
        if (*px == '/')
            py = px + 1;
    if (py)
        (void) strcpy(pn, py);
    return(strlen(pn));
}

```

**Table 3N**

```

/*
 * cleanup() -- cleanup any tmp file
 * getseq() -- read in seq, set dna, len, maxlen
 * g_malloc() -- calloc() with error checkin
 * readjumps() -- get the good jumps, from tmp file if necessary
 * writejumps() -- write a filled array of jumps to a tmp file: nw()
 */
#include "nw.h"
#include <sys/file.h>
char    *jname = "/tmp/homgXXXXXX";          /* tmp file for jumps */
FILE     *fj;
int      cleanup();                          /* cleanup tmp file */
long     lseek();
/*
 * remove any tmp file if we blow
 */
cleanup(i)                                  cleanup
{
    int      i;
    if (fj)
        (void) unlink(jname);
        exit(i);}
/*
 * read, return ptr to seq, set dna, len, maxlen
 * skip lines starting with ';', '<', or '>'
 * seq in upper or lower case
 */
char    *
getseq(file, len)                          getseq
{
    char    *file;      /* file name */
    int     *len;       /* seq len */

    char     line[1024], *pseq;
    register char *px, *py;
    int      natgc, tlen;
    FILE     *fp;
    if ((fp = fopen(file, "r")) == 0) {
        fprintf(stderr, "%s: can't read %s\n", prog, file);
        exit(1);
    }
    tlen = natgc = 0;
    while (fgets(line, 1024, fp)) {
        if (*line == ';' || *line == '<' || *line == '>')
            continue;
        for (px = line; *px != '\n'; px++)
            if (isupper(*px) || islower(*px))
                tlen++;
    }
    if ((pseq = malloc((unsigned)(tlen+6))) == 0) {
        fprintf(stderr, "%s: malloc() failed to get %d bytes for %s\n", prog, tlen+6, file);
        exit(1);
    }
    pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';

```

**Table 30**

...getseq

```

py = pseq + 4;
*len = tlen;
rewind(fp);
while (fgets(line, 1024, fp)) {
    if (*line == ';' || *line == '<' || *line == '>')
        continue;
    for (px = line; *px != '\n'; px++) {
        if (isupper(*px))
            *py++ = *px;
        else if (islower(*px))
            *py++ = toupper(*px);
        if (index("ATGCU", *(py-1)))
            natgc++;
    }
    *py++ = '\0';
    *py = '\0';
    (void) fclose(fp);
    dna = natgc > (tlen/3);
    return(pseq+4);
}
char *
g_calloc(msg, nx, sz)                                g_calloc
{
    char *msg; /* program, calling routine */
    int nx, sz; /* number and size of elements */

    char *px, *calloc();
    if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
        if (*msg) {
            fprintf(stderr, "%s: g_calloc() failed %s (n=%d, sz=%d)\n", prog, msg, nx, sz);
            exit(1);
        }
    }
    return(px);
}
/*
 * get final jmps from dx[] or tmp file, set pp[], reset dmax: main()
 */
readjmps()                                            readjmps
{
    int fd = -1;
    int siz, i0, i1;
    register i, j, xx;
    if (fj) {
        (void) fclose(fj);
        if ((fd = open(jname, O_RDONLY, 0)) < 0) {
            fprintf(stderr, "%s: can't open() %s\n", prog, jname);
            cleanup(1);
        }
    }
    for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; ; i++) {
        while (1) {
            for (j = dx[dmax].ijmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--)
                ;

```

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**Table 3P**

...readjumps

```

    if (j < 0 && dx[dmax].offset && fj) {
        (void) lseek(fd, dx[dmax].offset, 0);
        (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
        (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
        dx[dmax].ijmp = MAXJMP-1;
    }

    else
        break;
}

if (i >= JMPS) {
    fprintf(stderr, "%s: too many gaps in alignment\n", prog);
    cleanup(1);
}

if (j >= 0) {
    siz = dx[dmax].jp.n[j];
    xx = dx[dmax].jp.x[j];
    dmax += siz;
    if (siz < 0) { /* gap in second seq */
        pp[1].n[i1] = -siz;
        xx += siz;
        /* id = xx - yy + len1 - 1
        */
        pp[1].x[i1] = xx - dmax + len1 - 1;
        gapy++;
        ngapy -= siz;
        /* ignore MAXGAP when doing endgaps */
        siz = (-siz < MAXGAP || endgaps)? -siz : MAXGAP;
        i1++;
    }
    else if (siz > 0) { /* gap in first seq */
        pp[0].n[i0] = siz;
        pp[0].x[i0] = xx;
        gapx++;
        ngapx += siz;
        /* ignore MAXGAP when doing endgaps */
        siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
        i0++;
    }
}
else
    break;
}

/* reverse the order of jumps
*/
for (j = 0, i0--; j < i0; j++, i0--) {
    i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
    i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
}
for (j = 0, i1--; j < i1; j++, i1--) {
    i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
    i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
}

if (fd >= 0)
    (void) close(fd);

if (fj) {
    (void) unlink(jname);
    fj = 0;
    offset = 0;
}
}

```

**Table 3Q**

```

/*
 * write a filled jmp struct offset of the prev one (if any): nw()
 */
writejumps(ix)                                writejumps
{
    int      ix;

    char      *mktemp();

    if (!fj) {
        if (mktemp(jname) < 0) {
            fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);
            cleanup(1);
        }
        if ((fj = fopen(jname, "w")) == 0) {
            fprintf(stderr, "%s: can't write %s\n", prog, jname);
            exit(1);
        }
    }
    (void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);
    (void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);
}

```



**What is claimed:**

1. A method of enhancing, stimulating or potentiating the differentiation of T-cells into the Th2 subtype instead of the Th1 subtype, comprising contacting said T-cells with an effective amount of a TCCR antagonist.  
5
2. The method of claim 1, wherein the enhancing, stimulating or potentiating occurs in a mammal and the effective amount is a therapeutically effective amount.  
10
3. A method of treating a Th1-mediated disease in a mammal comprising administering to said mammal a therapeutically effective amount of a TCCR polypeptide antagonist.
4. The method of claim 3, wherein the Th1-mediated disease is selected from the group consisting of autoimmune inflammatory disease and allograft rejection.  
15
5. The method of claim 4, wherein the autoimmune inflammatory disease is selected from the group consisting of allergic encephalomyelitis, multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune uveoretinitis, inflammatory bowel disease and autoimmune thyroid disease.  
20
6. The method of claim 3, wherein the antagonist is a small molecule.
7. The method of claim 3, wherein the antagonist is an antisense oligonucleotide.
8. The method of claim 7, wherein the oligonucleotide is RNA.  
25
9. The method of claim 7, wherein the oligonucleotide is DNA.
10. The method of claim 3, wherein the antagonist is a TCCR variant lacking biological activity.  
30
11. The method of claim 3, wherein the antagonist is a monoclonal antibody.
12. The method of claim 11 wherein the antibody has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues.  
35
13. The method of claim 3 wherein the antagonist is an antibody fragment or a single-chain antibody.
14. The method of claim 3 wherein the antagonist is a TCCR ligand.

15. A method of preventing, inhibiting or attenuating the differentiation of T-cells into the Th2 subtype, comprising the administration of an effective amount of a TCCR polypeptide or agonist thereof.
- 5 16. The method of claim 15, wherein the preventing, inhibiting or attenuating occurs in a mammal and the effective amount is a therapeutically effective amount.
17. A method of treating a Th2-mediated disease in a mammal comprising the administration to said mammal a therapeutically effective amount of a TCCR polypeptide or agonist.
- 10 18. The method of claim 17, wherein the Th2-mediated disease is selected from the group consisting of: infectious diseases and allergic disorders.
- 15 19. The method of claim 18, wherein the infectious disease is selected from the group consisting of: *Leishmania major*, *Mycobacterium leprae*, *Candida albicans*, *Toxoplasma gondi*, respiratory syncytial virus and human immunodeficiency virus.
20. The method of claim 18, wherein allergic disorder is selected from the group consisting of: asthma, allergic rhinitis, atopic dermatitis and vernal conjunctivitis.
- 20 21. The method of claim 15, wherein the agonist is a small molecule.
22. The method of claim 15, wherein the agonist is a TCCR variant having biological activity.
23. The method of claim 15, wherein the agonist is a monoclonal antibody.
- 25 24. The method of claim 23, wherein the antibody has nonhuman complementarity determining region (CDR) residues and human framework region (FR) residues.
25. The method of claim 15, wherein the agonist is an antibody fragment or a single-chain antibody.
- 30 26. The method of claim 15, wherein the agonist is a stable TCCR ECD.
27. A method for determining the presence of a TCCR polypeptide in a cell, comprising exposing the cell to an anti-TCCR antibody and measuring binding of the antibody to the cell, wherein binding of the antibody to the cell is indicative of the presence of TCCR polypeptide.
- 35 28. A method of diagnosing a Th1-mediated or Th2-mediated disease in a mammal, comprising detecting the level of expression of a gene encoding a TCCR polypeptide (a) in a test sample of tissue cells obtained from

the mammal, and (b) in a control sample of known normal tissue cells of the same cell type, wherein a lower expression level in the test sample as compared to the control sample indicates the presence of a Th2-mediated disorder and a higher expression level in the test sample as compared to the control sample indicates the presence of a Th1-mediated disorder.

5

29. A method for identifying a compound capable of inhibiting the expression of a TCCR polypeptide comprising contacting a candidate compound with the polypeptide under conditions and for a time sufficient to allow these two components to interact.

10 30. The method of claim 29, wherein the candidate compound is immobilized on a solid support.

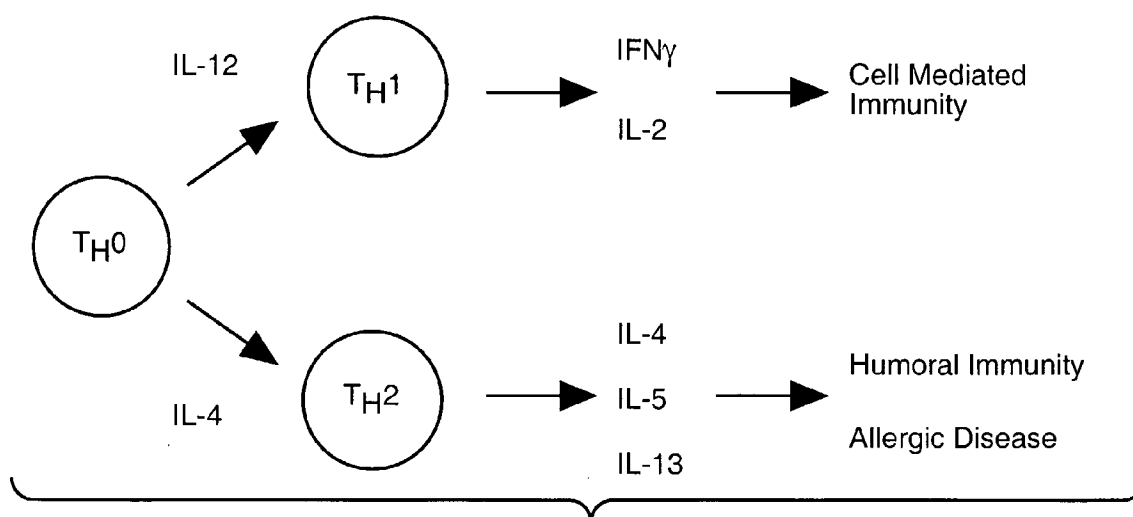
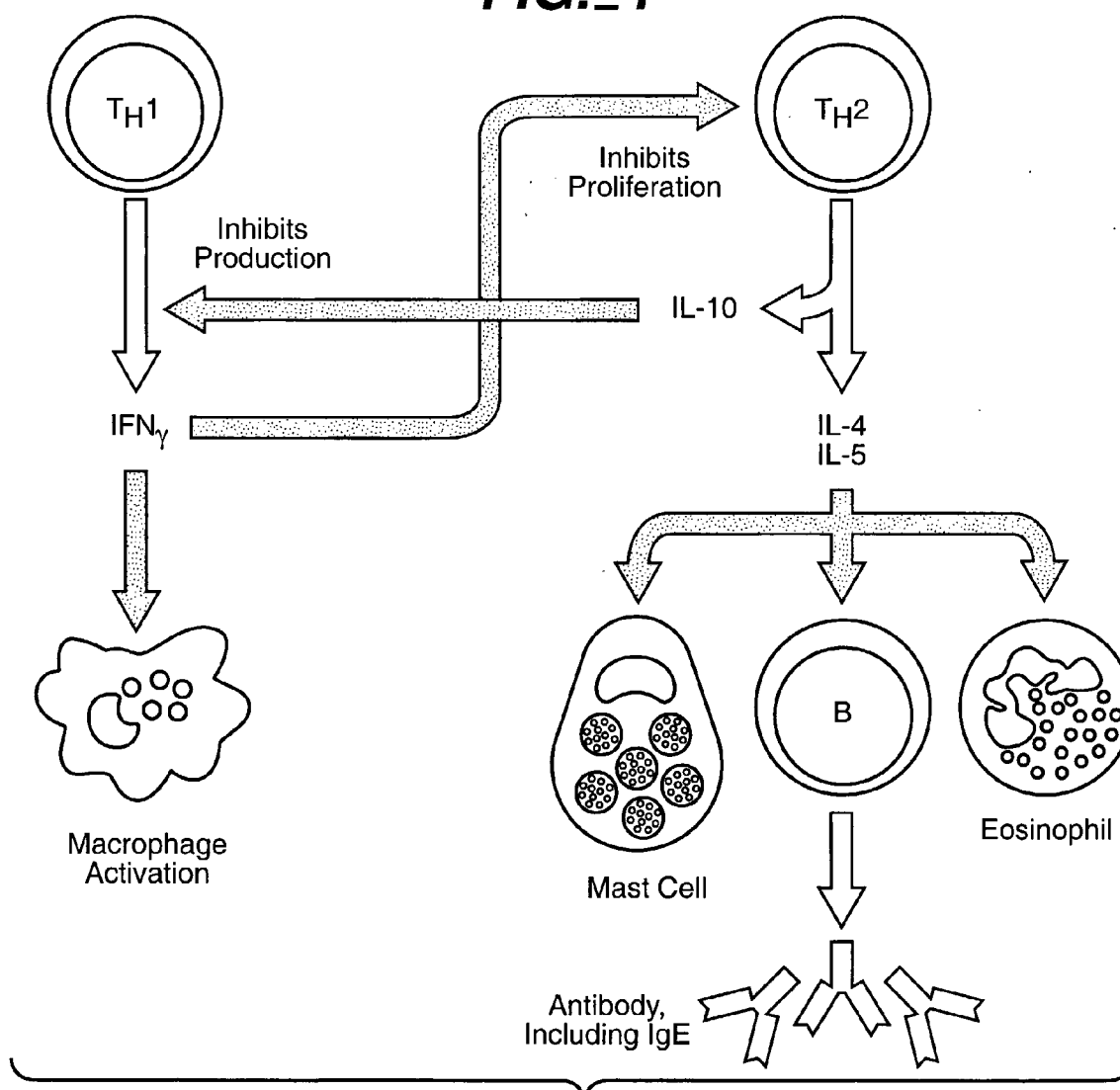
31. The method of claim 30, wherein the non-immobilized component carries a detectable label.

15 32. A method for identifying a compound capable of inhibiting a biological activity of a TCCR polypeptide comprising contacting a candidate compound with the polypeptide under conditions and for a time sufficient to allow these two component to interact.

33. The method of claim 32, wherein the candidate compound is immobilized on a solid support.

20 34. The method of claim 33, wherein the non-immobilized component carries a detectable label.

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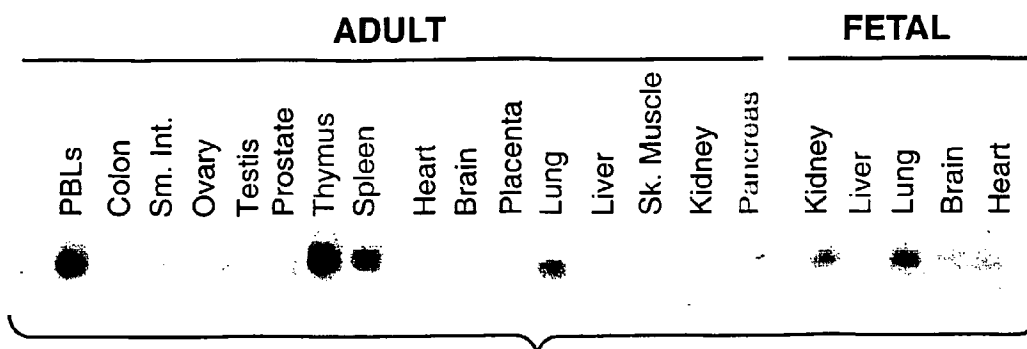
**FIG.\_1****FIG.\_2**

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MRGGRGAPFWLWPLPKLALLPLLWVLFQRTRPQGSAGPLQCYGVGPLGDLNCSWEPLGD  
 LGAPSELHLQSQKYRSNKTQTVAVAAGRSWVAIPREQLTMSDKLLVWGTKAGQPLWPPV  
 FVNLETQMKPNAPRLGPDVDFSEDDPLEATVHWAPPTWPSHAVLICQFHYRRCQEAAWT  
 LLEPELKTIPLTPVEIQDLELATGYKVYGRCRMEKEEDLWGEWSPILSFQTPPSAPKDV  
 WVSGNLCGTPGGEEPLLLWKAPGPCVQVSYKVWFWVGGRELSPEGITCCCCSLIPSGAEW  
 ARVSAVNATSWEPLTNLSLVCLDSASAPRSVAVSSIAGSTELLVTWQPGPGEPLEHVVD  
 WARDGDPLEKLNWVRLPPGNLSALLPGNFTVGVYPYRITVTAVSASGLASASSVWGFREE  
 LAPLVGPTLWRLQDAPPGTPAIAWGEVPRHQLRGHLTHYTLCAQSGTSPSVCMNVSGNT  
 QSVTLPLDLPWGPCELWVTASTIAGQGGPPGPILRLHLPDNTLRWKVLPGLFLWGLFLLG  
 CGLSLATSGRCYHLRHKVLPRWVWEKVPDPANSSSGQPHMEQVPEAQPLGDLPILEVEE  
 MEPPPVMESSQPAQATAPLDSGYEKHFLPTPEELGLLGPPRPQVLA

**FIG.\_3**

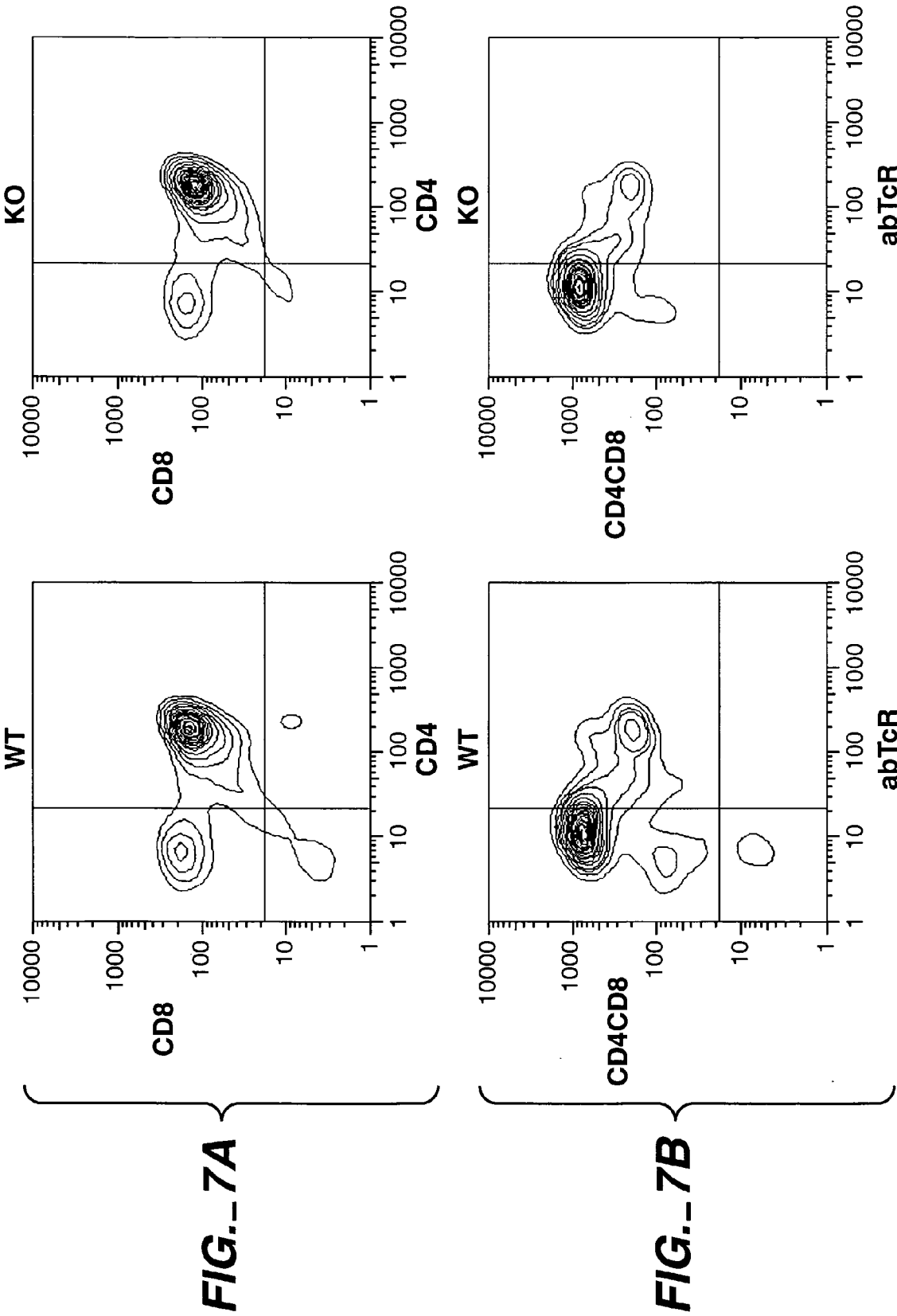
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 LYHQSQKYHPNRVWEVKVPSKQSWVTIPREQFTMADKLLIWGTQKGRPLWSSSVNLETQ  
 MKPDTPOIFSQVDISEEATLEATVQWAPPVWPPQKALTCQFRYKECQAEAWTRLEPQLKT  
 DGLTPVEMQNLEPGTCYQVSGRCQVENGYPWGEWSSPLSFQTPFLDPEDVWVSGTVCETS  
 GKRAALLVWKDPRPCVQVTTYTVWFGAGDITTTQEEVPCKSPVPAWMEWAVVSPGNSTSW  
 VPPTNLSLVCLAPESAPCDVGVSADGSPGIKVTWKQGTRKPLEYVVDWAQDGDSDLKLN  
 WTRLPPGNLSTLLPGEFKGGVPYRITVTAVYSGGLAAAPSVWGFREELVPLAGPAVWRLP  
 DDPPGTPVVAWGEVPRHQLRGQATHYTFICIQSRGLSTVCRNVSSQTQTATLPNLHSGSFK  
 LWVTVSTVAGQGGPPGDLHLPLDNRIRWKALPWFLSLWGLLLMGCGLSLASTRCLQARC  
 LHWRHKLPLQWIWVDPANSSNGQPYIKEVSLPQPPKDGPILEVEEVELQPVVESPKA  
 SAPIYSGYEKHFLPTPEELGLLV

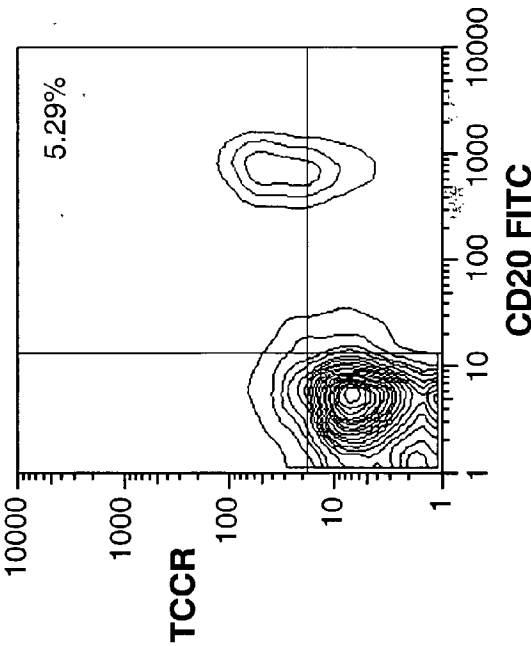
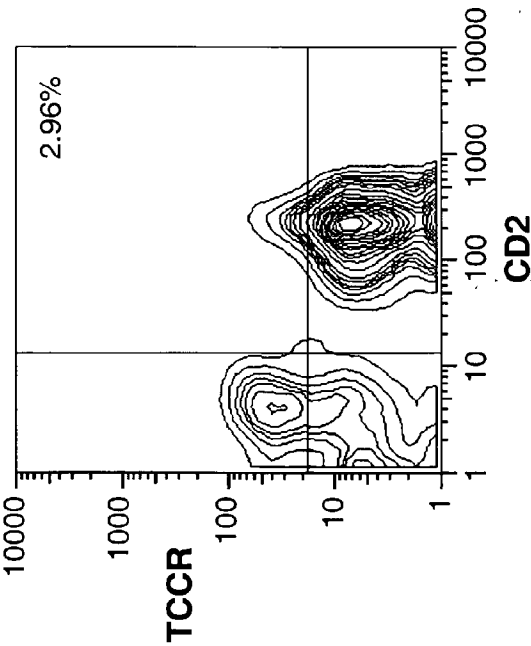
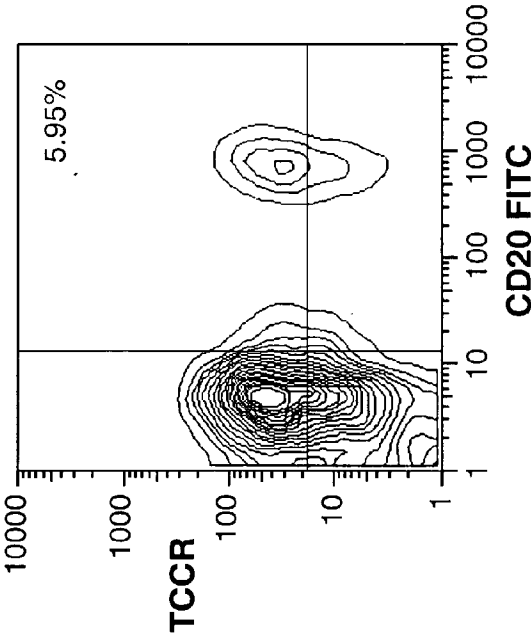
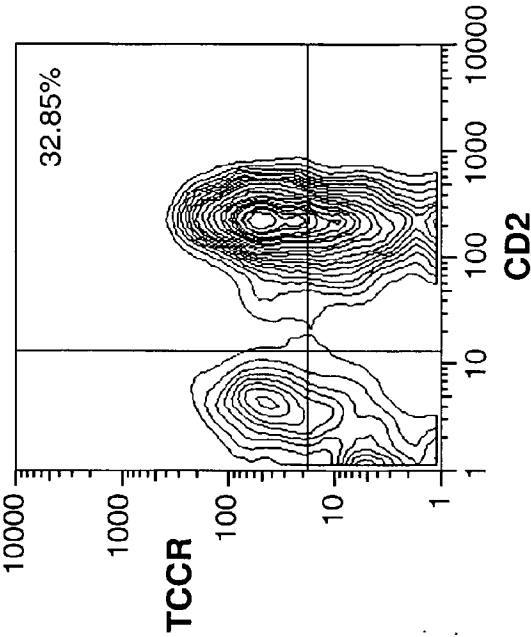
**FIG.\_4****FIG.\_6**

h-TCCR	1	MRGGRGAPFWLWPLPKLALLPLLWVLFQTRPQGSAGPLQCYGVGPIGLDL
m-TCCR	1	-----MNRRLVARLTPLLELLLSLSLMSLLLGTRPHGSPGLQCYSVGPIGLIL
h-TCCR	51	NCSWEPLGDLGAPSELHLLQSKYRSNKTQTVAAGRSWVAIPREQLLTMS
m-TCCR	46	NCSWEPLGDLLETPLVYHQSQKYHNNRVWEVKVPSKQSWVTIPREQFTMA
h-TCCR	101	DKLLVWGTAKAGQPLWPPVFNLETQMKPNAPRLGPDVDFFSEDDPLEATVH
m-TCCR	96	DKLLIWGTQKGRPLWSVSFNLETQMKPDTPQIFSQVDISEEATLEATVQ
h-TCCR	151	WAPPTWPSHKVLIICQFHYRRCCQEAATLLEPELKTIPLTPVEIQDLELAT
m-TCCR	146	WAPPVWPPQKALTCCQFHYRRCQEAATLLEPELKTIPLTPVEIQDLELAT
h-TCCR	201	GKYYGRCRMEKEEDLWGEWSPILSFQTPPSAPKDVWVSGNLCGTPGGEE
m-TCCR	195	CYQVSGRCQVENGYP-WGEWSPILSFQTPFLDEPEDVWVSGTVCETSGKRA
h-TCCR	251	PLLWKAPGPCVQVSYKWWFVWGGRELSPGEGITCCCSLIPSGAEWARVSA
m-TCCR	245	ALLVWKDPRPCVQVITYVWFAGDITTTQEEVPCCKSPVPAWMEWAVVSP
h-TCCR	301	VNATSWEPILTNSLVCLDSASAPRSVAVSSIAGSTELLVTWQPGGEPLE
m-TCCR	295	GNSTSWVPPNTNSLVCLAPESAPCDVGSSADGSPGKVTWKQGTCKPPE
h-TCCR	351	HVVDWARDDGDPLEKLNWVRLPFGNLSALLPGNFTVGPYRITVTAVSASG
m-TCCR	345	YVVDWAQDGDSDLKLNWTRLPPGNLSLTLLPGFEKGGVPYRITVTAVYSGG
h-TCCR	401	LASASSVWGFREELAPLVGPTLWRLQDAPPGTPAIWGEVPRRHQLRGHLT
m-TCCR	395	LAAAPS VWGFREELVPLAGPAVWRLPDDPPGTTPVWAWGEVPRRHQLRQAT
h-TCCR	451	HYTLCAQSGTSPSVCMNVSGNTQSVTLPPDLLPWGPCCELWVTASTIAGQGGPP
m-TCCR	445	HYTFCIQSRGLSTVCRNVSSQTQTATLPLNLLHSGSFKLWVTVSTVAGQGGPP
h-TCCR	501	GPIRLRLHLPDNTLLRWKVLPGILFLFWGLFLLGCGLSLATS---GRCYHLR
m-TCCR	495	GPDLSLHLPDNNRIRWKALPWFLLSLWGLLLMGCGLSLASTRCLQARCLHWR
h-TCCR	547	HKVLPRLWVWEKVPDPANSSSGQPHMEQVPEAQPLGDLPILEVEEEMEPVPV
m-TCCR	545	HKLLPQWIWERVDPDPANSSNSGQPYIKEVSLPQPPKDGPILEVEEVELQPV
h-TCCR	597	MESQPAQAATAPLDSGYEKHFLLPTPEELGILLGPPRPQVLA
m-TCCR	595	VES---PKASAPIYSGYEKHFLLPTPEELGILLV

Box 1

FIG. 5



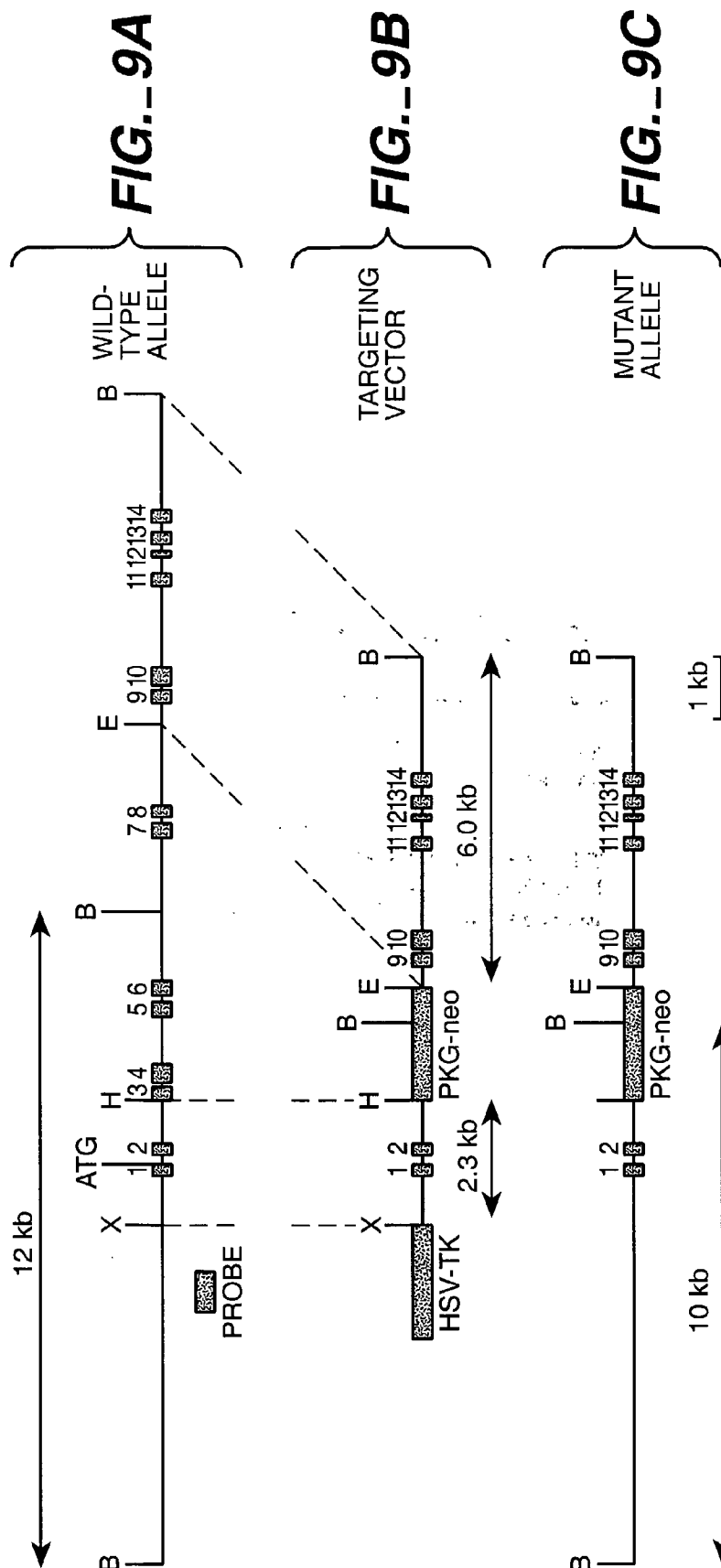


**FIG. 8A**

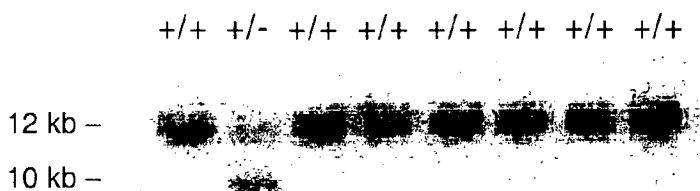
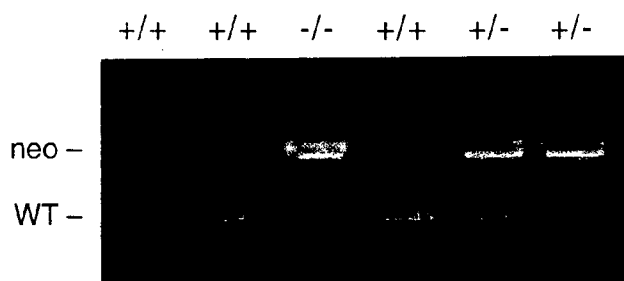
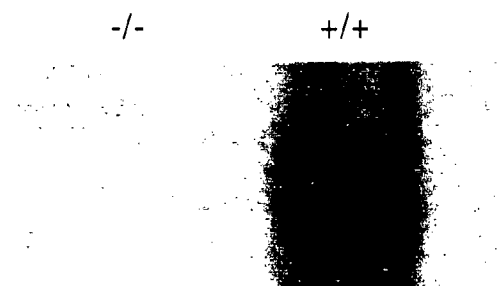
**FIG. 8B**



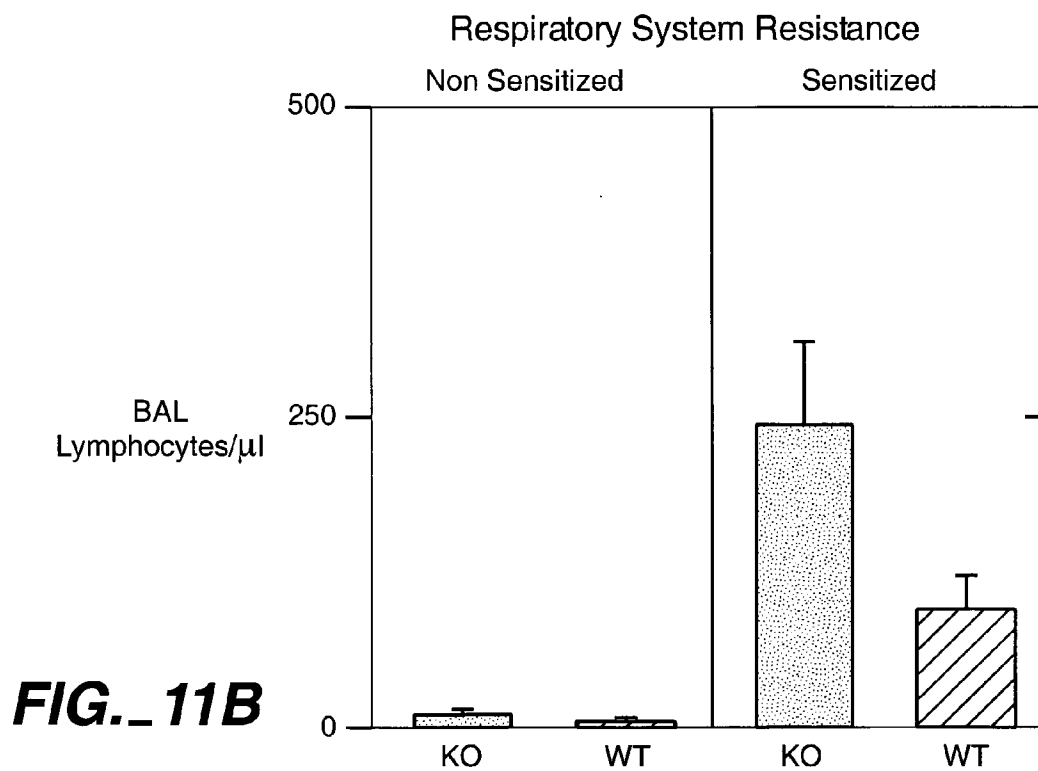
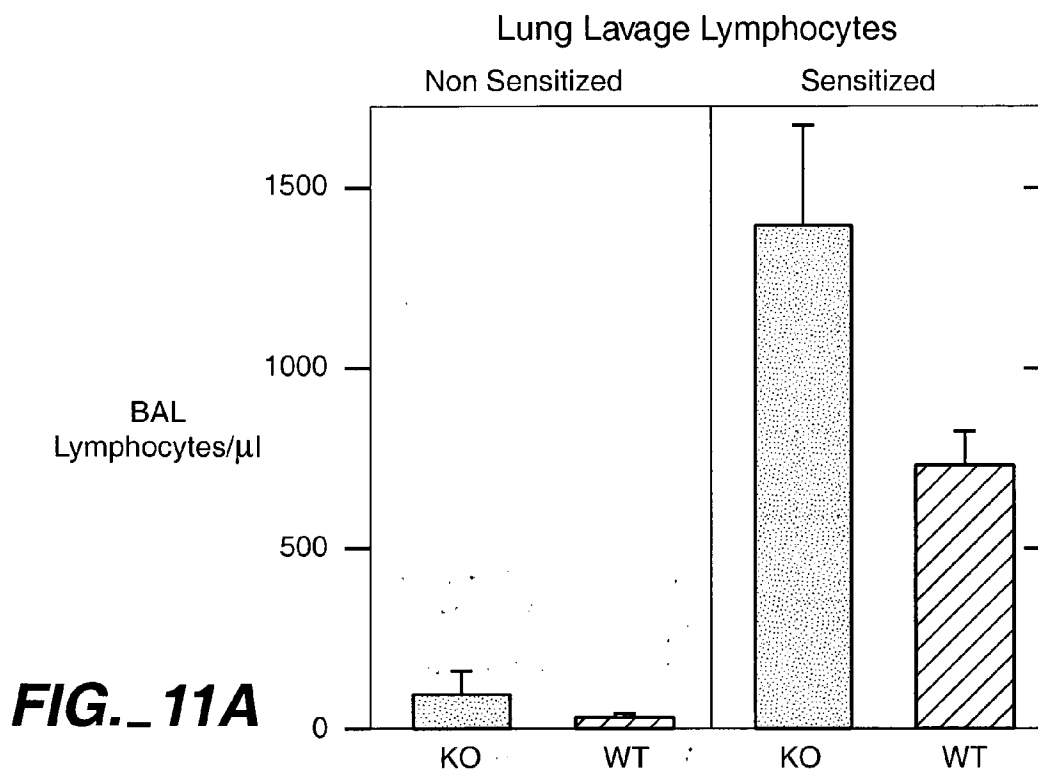
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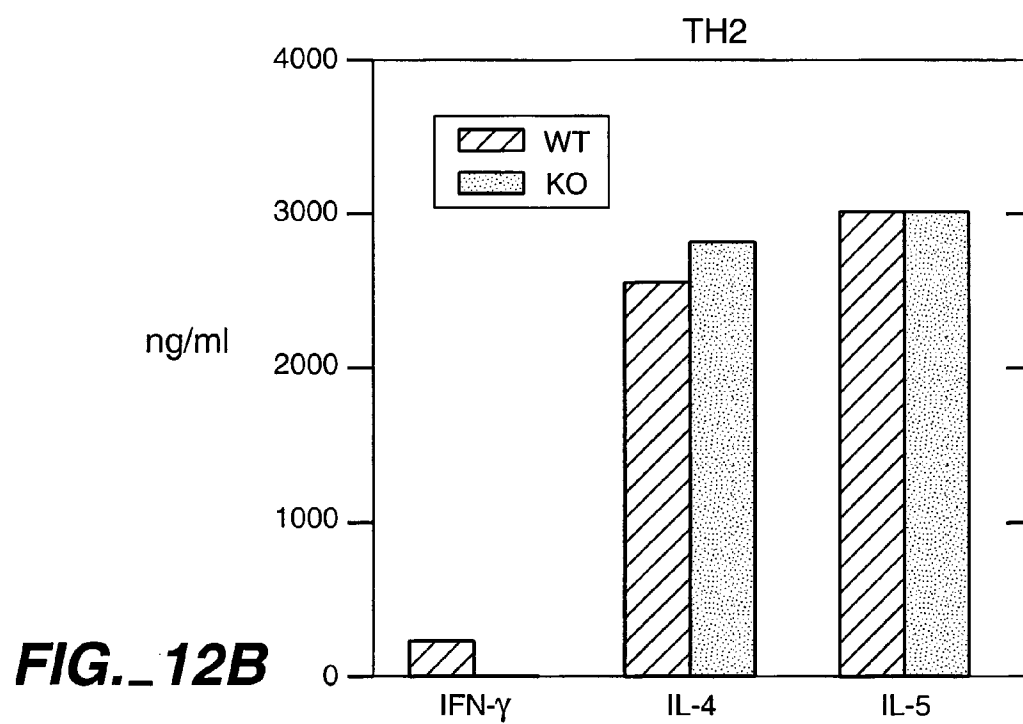
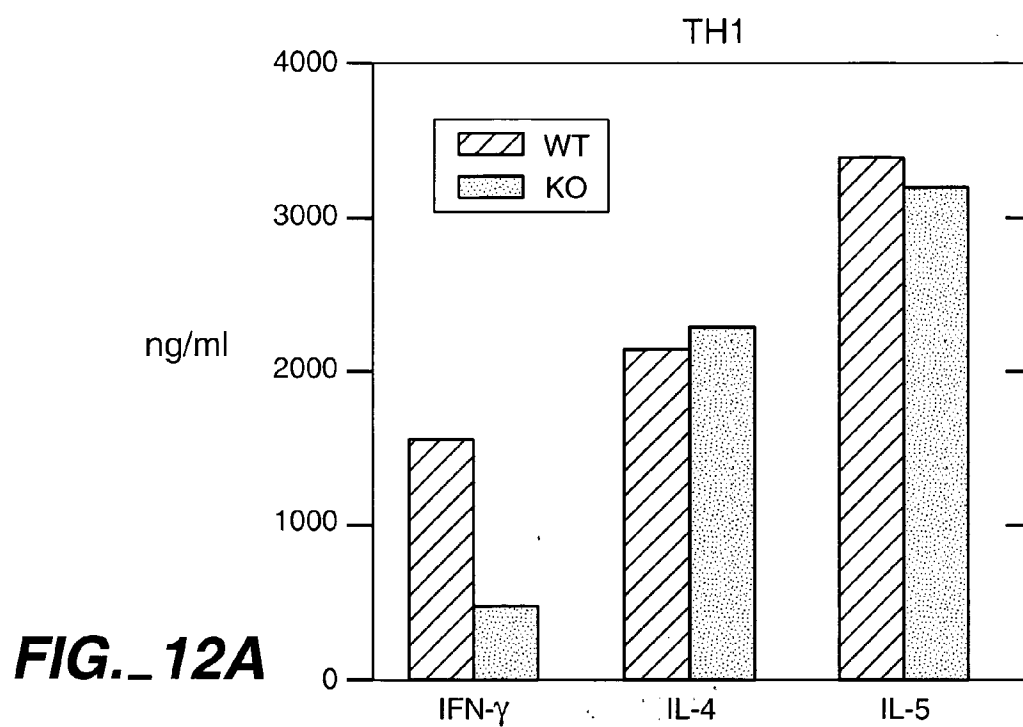
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**FIG.\_10A****FIG.\_10B****FIG.\_10C**

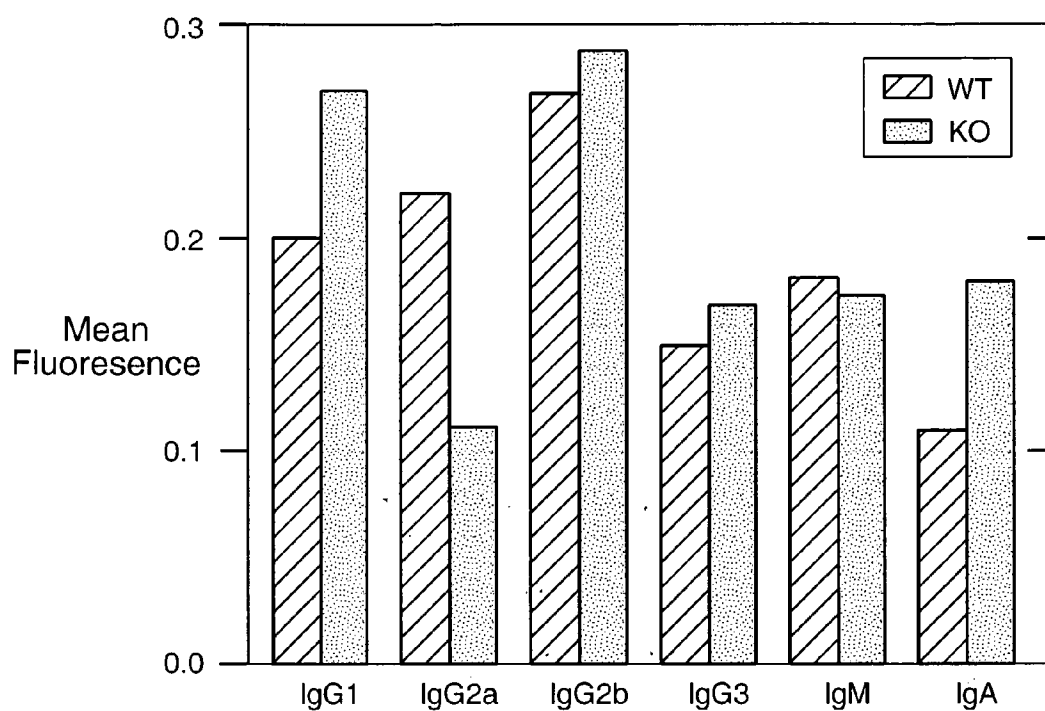
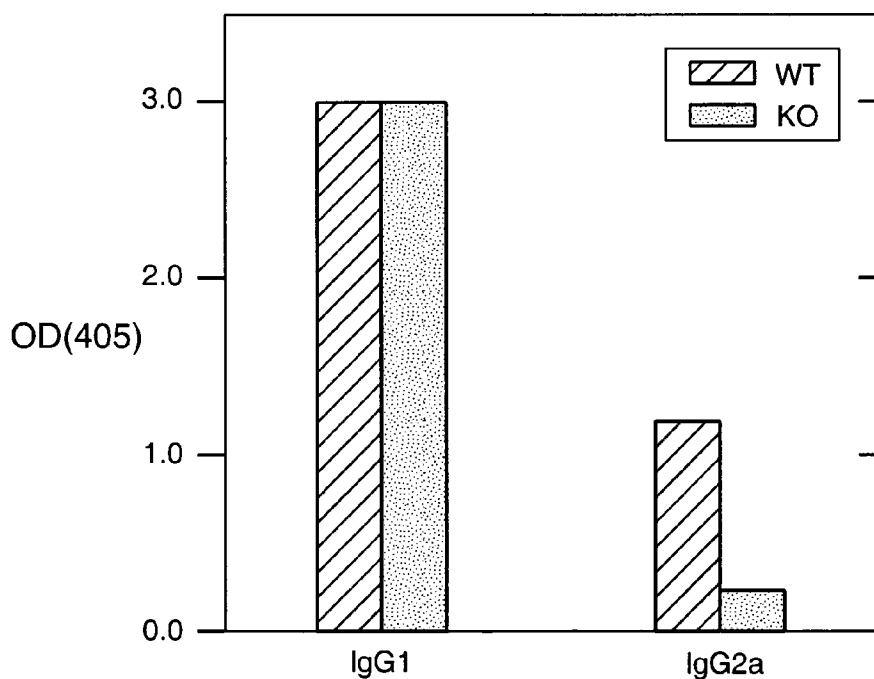
8 / 14

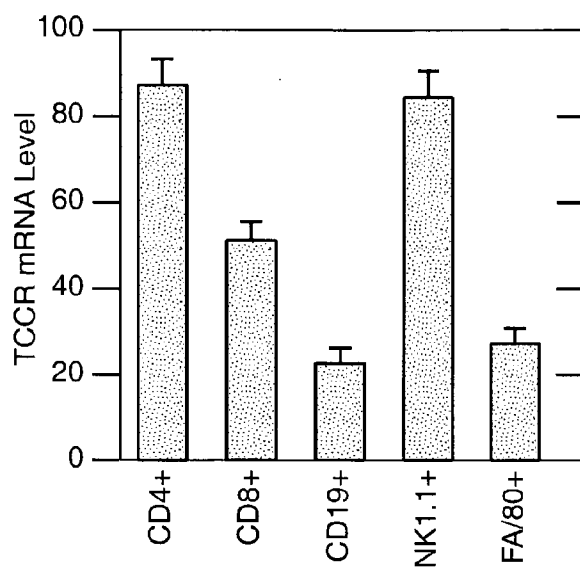
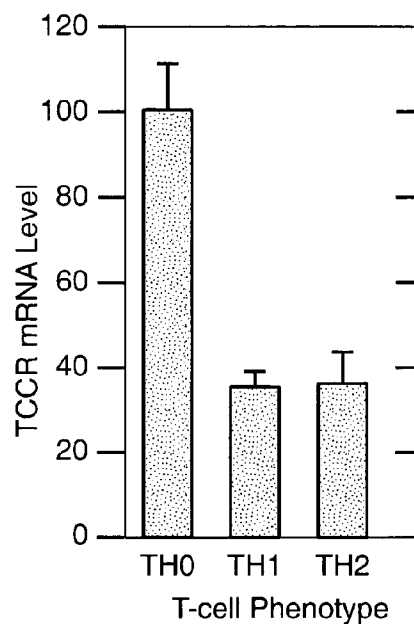
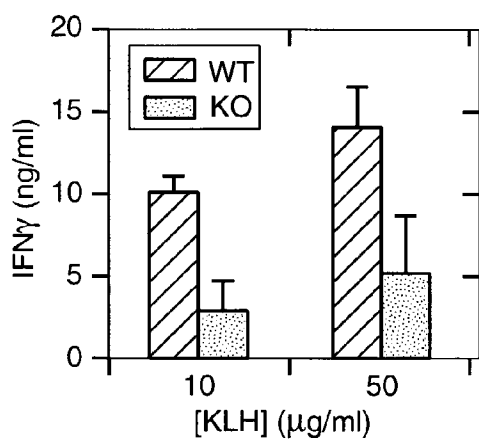
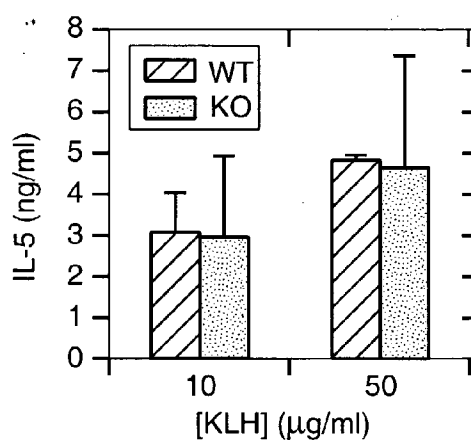
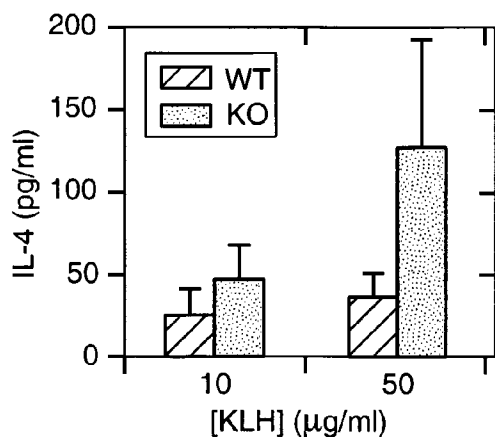
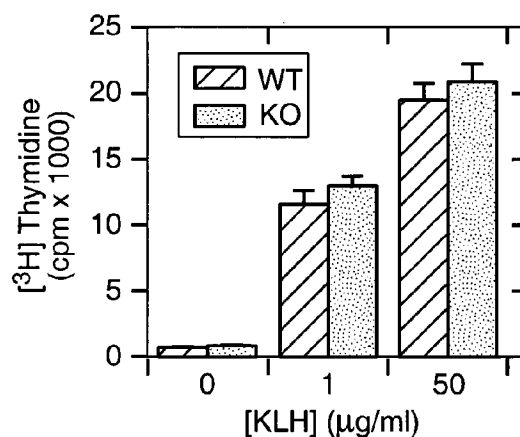


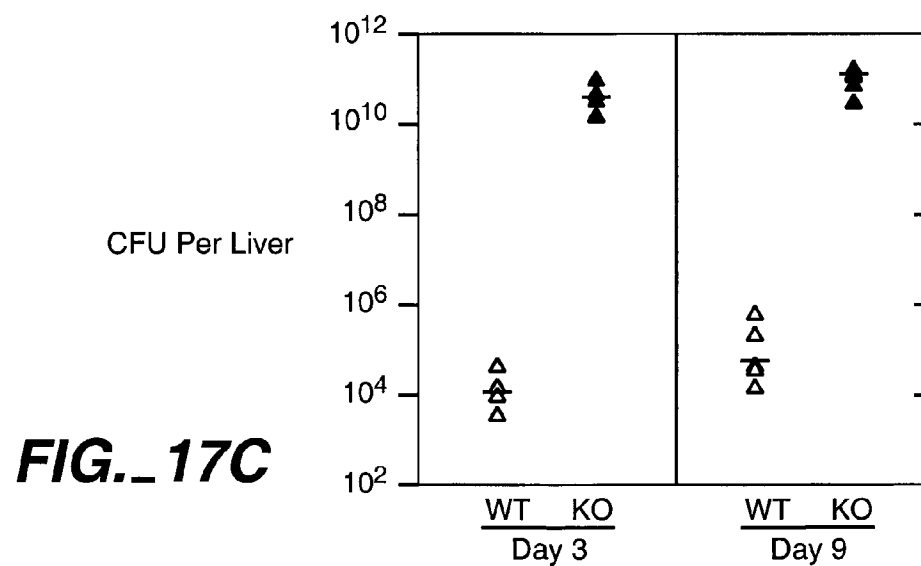
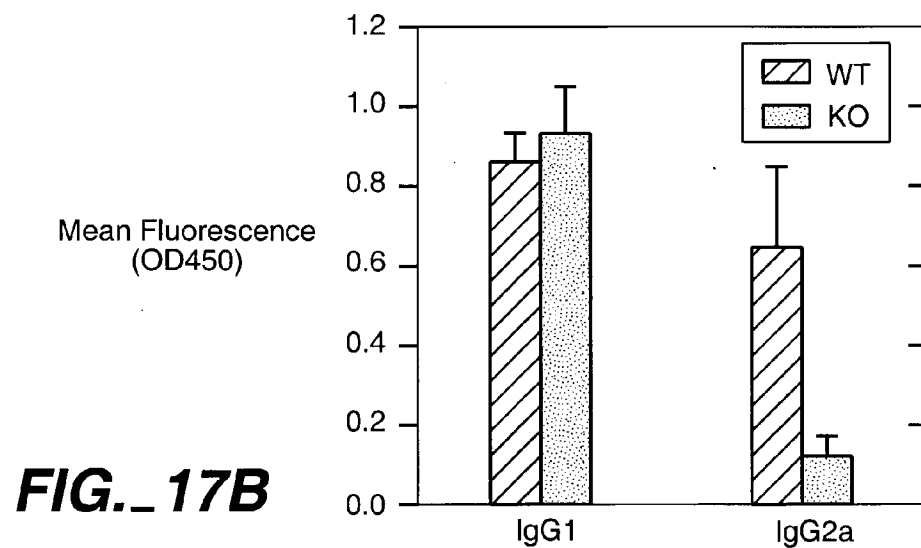
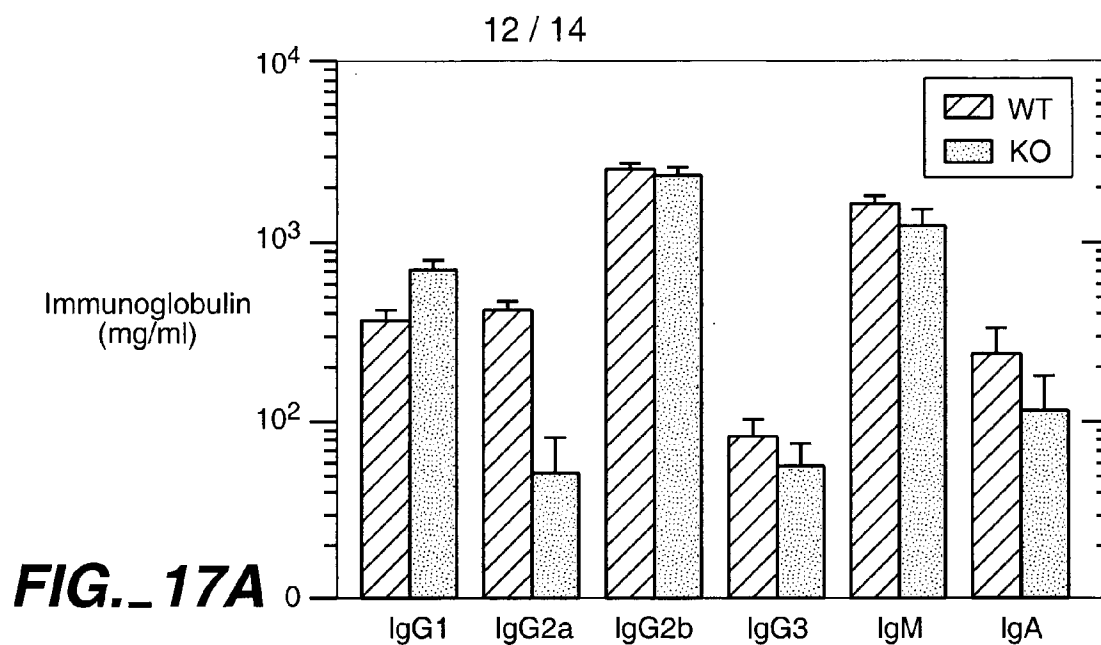
9 / 14

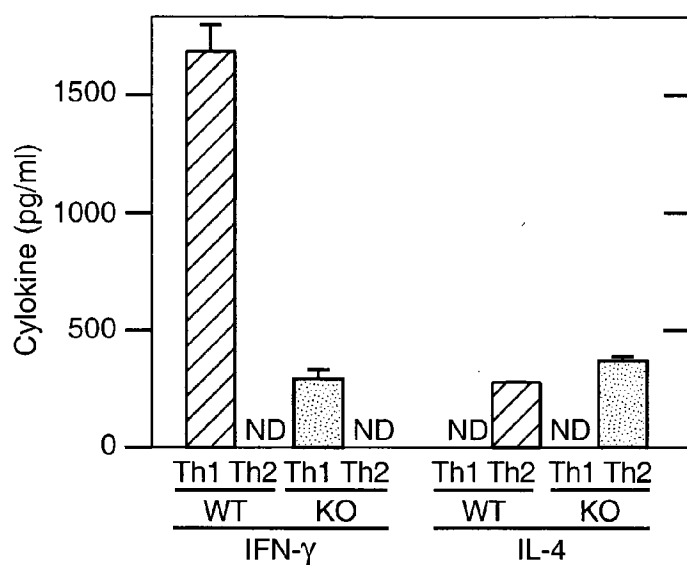
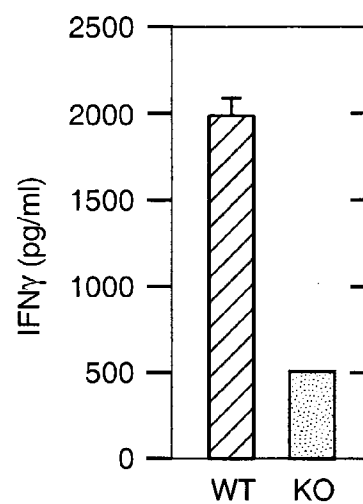
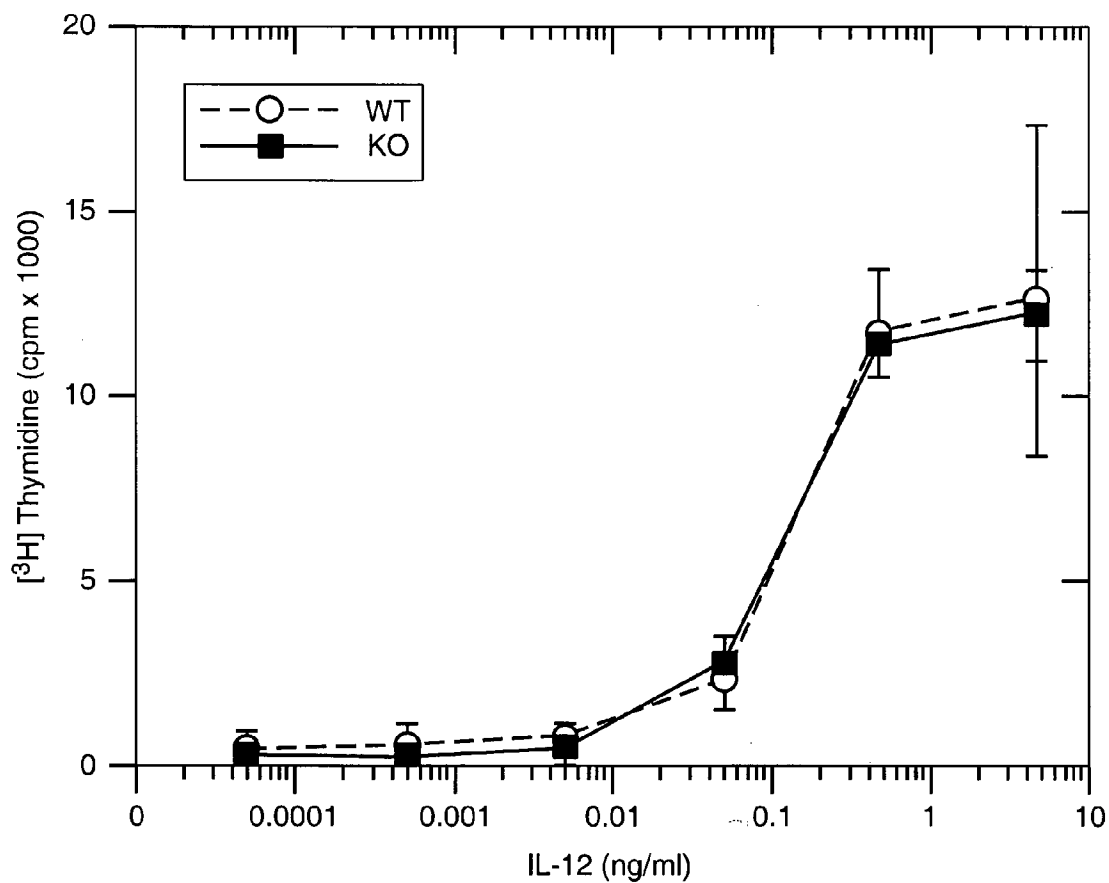


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**FIG. 13****FIG. 14**

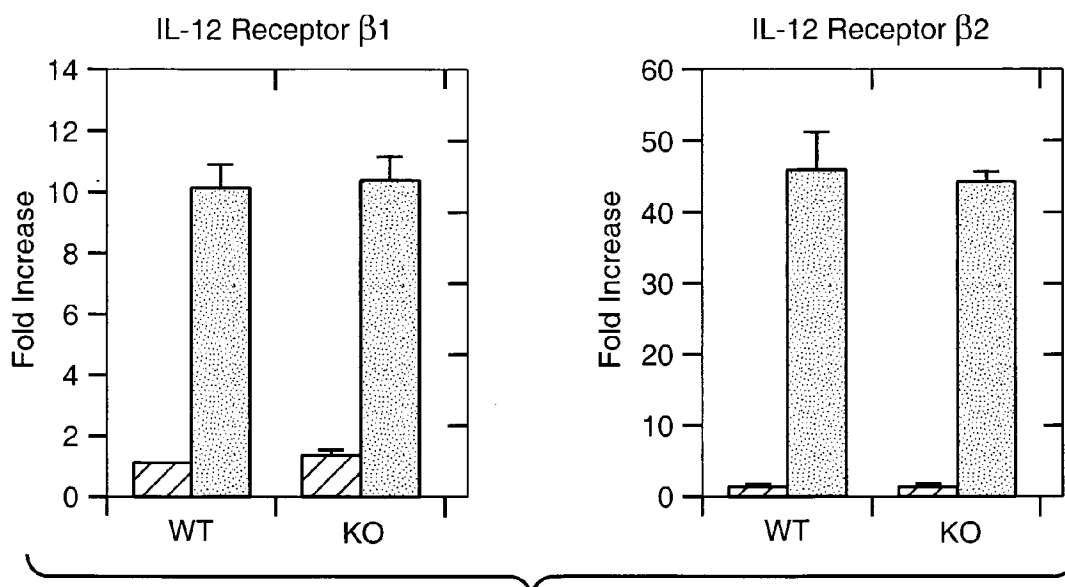
**FIG.\_15A****FIG.\_15B****FIG.\_16A****FIG.\_16C****FIG.\_16B****FIG.\_16D**



**FIG. 18A****FIG. 18B****FIG. 18C**



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**FIG.\_18D**

Primer / Probe	Sequence	SEQ ID NO:
mTCCR, sense, Taqman	TGGTCTCTCCTGGCAACAGC	5
mTCCR, as, Taqman	AGCCAAGCACACCAGAGACA	6
mTCCR, Taqman probe	CAGCTGGGTGCCTCCCACCAA	7
mRPL19, sense, Taqman	ATCCGCAAGCCTGTGACTGT	8
mRPL19, as, Taqman	TCGGGCCAGGGTGTTTTT	9
mRPL19, Taqman probe	TTCCCGGGCTCGTTGCCG	10
mIL12Rb1, sense, Taqman	TCGCGTCTCTGGGAAGCT	11
mIL12Rb1, as, Taqman	TTTAAGCCAATGTATCCGAGACTG	12
mIL12Rb1, Taqman probe	CGCCAGCGTCCTCCTCGTGG	13
mIL12Rb2, sense, Taqman	CAAGCATTTGCATCGCTATCA	14
mIL12Rb2, as, Taqman	AATGCCTTTTGCCGGAAGT	15
mIL12Rb2, Taqman probe	ACGAATTGAGAACGTGCCCACCGT	16

**FIG.\_19**

## Sequence Listing

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 De Sauvage, Frederic  
 Grewal, Iqbal  
 Gurney, Austin L

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 Phe His Tyr Arg Arg Cys Gln Glu Ala Ala Trp Thr Leu Leu Glu  
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 200 205 210  
 Glu Lys Glu Glu Asp Leu Trp Gly Glu Trp Ser Pro Ile Leu Ser  
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	Ile	Ala	Gly	Ser	Thr	Glu	Leu	Leu	Val	Thr	Trp	Gln	Pro	Gly	Pro	
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		50	55	60
	Leu Tyr His Gln	Ser Gln Lys Tyr His	Pro Asn Arg Val Trp	Glu
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	Asp Gly Leu Thr	Pro Val Glu Met Gln	Asn Leu Glu Pro Gly	Thr
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	Gln Ser Arg Gly Leu Ser Thr Val Cys Arg Asn Val Ser Ser Gln	455	460	465
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